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**PROCESSED PLANT
PROTEIN FOODSTUFFS**

PROCESSED PLANT PROTEIN FOODSTUFFS

Edited by

AARON M. ALTSCHUL

*United States Department of Agriculture
New Orleans, Louisiana*

See Chap 3

See Chap 22 (II) pp 620-



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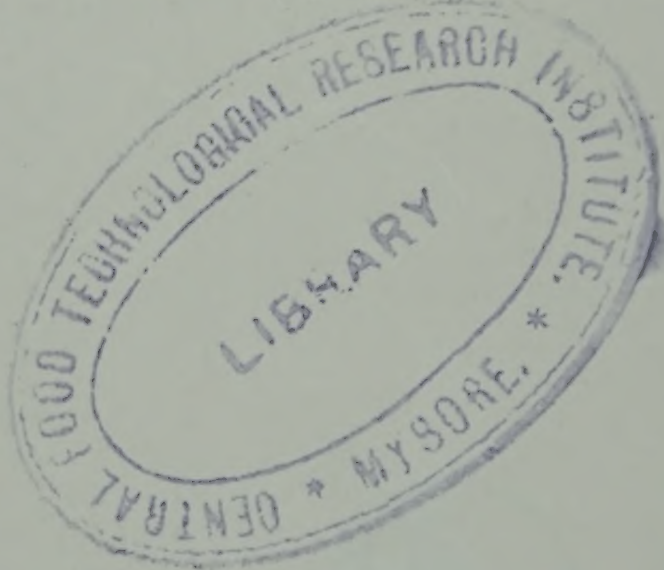
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PREFACE

This volume tells about the sources, production, and processing of plant protein foodstuffs and about how they are used and why they are used. It is addressed to nutritionists, medical scientists, and students of proteins; to government and health authorities who seek to solve the problem of feeding a growing world population; and to commercial producers and consumers of plant protein foodstuffs.

The general aim of this work is to present information needed alike by growers, producers, and users, and to bridge the gaps between different kinds of knowledge and interest. An expert in general protein chemistry may want more special information about plant proteins. The expert in nutrition may want a background of knowledge of plant protein raw materials and of how they are processed and used. The processor may want to know more about feeding and nutritional problems. I hope that all who turn to the subject of plant protein foodstuffs will find portions of this volume interesting and informative; if gaps in knowledge become evident, as surely they will, perhaps this will stimulate research to seek out additional information.

This book is organized to present both a broad view of the problems and practices in the manufacture and use of processed plant protein foodstuffs and a detailed discussion of the major protein sources. Part I deals with broad aspects of plant proteins and their utilization in animal and human foodstuffs; Part II deals with the individual commodities. The fundamentals of nutrition as applied to plant products and the chemical changes that take place during processing are treated extensively. And this is followed by examples of practical application of these principles to the various commodities. Major emphasis is on present-day practice and uses; these are presented from an international viewpoint. But trends and the possibilities for the future are also discussed.

To accomplish the varied purposes of this book, the minds of many authors, each expert in his own field, were brought together. Thus a maximum amount of first-hand information has been provided, but with the unavoidable disadvantage of differences in style and method of presentation. Wherever possible an attempt has been made to present a uniform approach and treatment.

A certain amount of overlapping has been the result of having many

authors and of treating the subject matter from the broad, as well as the detailed, point of view. There is also the danger of differences of opinion arising in treatments of similar subjects by the various authors. We have sought to eliminate the differences in fact where they can be eliminated and confine those that occur to differences in approach and interpretation. Overlap has been kept as small as possible; it has been retained only for the sake of completeness of each individual treatment and for the convenience of the reader.

There has been vast improvement in methods for amino acid analysis; when applied to isolated proteins, a high degree of accuracy has been attained. Analysis for amino acids in seeds and in protein products is less certain; the amino acid contents of the same protein foodstuff as reported by various investigators are not always in agreement. We have, therefore, presented, wherever possible, several sets of analyses from the literature for each individual protein material so that the reader may appreciate the extent of variation.

This is a collaborative volume; many, too many to list them individually, contributed ideas, information, and criticism. I am grateful to R. Antonissen of Buenos Aires, B. S. Kulkarni of Hyderabad (India), K. S. Markley of Rio de Janeiro, J. Duckworth of Bucksburn (Scotland), Hiroshi Tamiya of Tokyo and J. A. van Veen of the Food and Agriculture Organization of the United Nations (Rome) for their comments and information needed to strengthen the international character of this volume; to Mrs. Evald Skau for invaluable help with the bibliographies; to my colleagues at the Southern Utilization Research and Development Division and the Director, C. H. Fisher, for advice and review; to M. L. Anson and to the staff of Academic Press for many helpful suggestions; and to my secretary, Mrs. Jean Guercia, for her wholehearted help and interest which made detail work so much less a chore and a more pleasant experience throughout. Finally, I acknowledge my debt to my wife, Ruth, whose encouragement and understanding, as well as help in so many details, provided me with the courage to complete this work.

AARON M. ALTSCHUL

New Orleans, Louisiana
March 13, 1958

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CHAPTER 1

INTRODUCTION

M. L. ANSON AND A. M. ALTSCHUL

I. CONTENTS OF THE BOOK

This book deals with commercial processed plant protein foodstuffs for animals and man. Although many such products will be described, the great bulk of protein comes from the so-called meals or cakes remaining after removal of oil from three oilseeds: soybean, cottonseed, and peanut. Fortunately, these meals, which were originally by-products of oil pressing, have two great virtues as protein feeds: they are concentrated in protein, and their protein-nutritional values for non-ruminants range from fairly good to quite good. Grains and forage grasses, major plant protein foodstuffs with which this book deals only incidentally, are both less rich in protein than the oilseed meals. Moreover, grain proteins, in general, have relatively low protein-nutritional value for non-ruminants. And the grasses which do contain good protein also contain a great deal of cellulose which non-ruminants cannot digest.

Although the total world-wide consumption of the three major oilseed meals exceeds by far the volume of other commercial processed vegetable protein foodstuffs, other vegetable protein foodstuffs are of great, and sometimes of predominant, importance in special regions and feeding situations. Linseed meal, for example, is a leading feed for ruminants in certain areas, and coconut oil and palm kernel meals are widely used as dairy feeds. Furthermore, some oilseed meals not used to the extent of the main oilseed meals are still used on such a large scale as to be of marked economic importance. Finally, some processed vegetable protein foodstuffs are of interest because of their special nutritive contributions beyond that of protein. Thus alfalfa meal and dried yeast are important sources of supplementary vitamins and other micronutrients.

Any discussion of protein foodstuffs must, of course, begin with a discussion of protein nutrition. In the narrow sense, protein nutrition

deals with the isolated protein as a source of amino acids for the animal's production of its own body protein. In the broader sense, protein nutrition deals with everything that makes a product possible and suitable for real-life consumption as a protein foodstuff: absence of undesirable substances or processing damage to the protein, the possibilities of practical supplementation, suitable concentration of protein, availability at an acceptable cost, pleasant taste, etc. Thus this book is concerned not only with the proteins as amino acid compounds, but also with the technology of the protein foodstuffs, with agricultural and industrial economics, with the tastes and choices of animals and men—indeed, with all the complex factors that influence the production and use of protein foodstuffs.

In this Introduction we shall take up, in a general way, some broad and often familiar considerations which cut across the special subjects of the individual chapters.

II. NUTRITION

Complexity of the nutritional problem. The practical nutritional value of a protein or of a protein foodstuff cannot be described in terms only of its composition and its digestibility. One must specify in addition what kind of an animal is eating the protein—ruminant or non-ruminant, mammalian or non-mammalian, infant or adult. And one must specify how the protein has been treated and supplemented, at what level the protein is being fed, and what other proteins are being fed. (See also Chapters 2, 7, 8, and 9.)

Ruminants vs. non-ruminants. It is common knowledge that ruminants can use protein foodstuffs which are not suitable for non-ruminants. First, non-ruminants must have certain so-called essential amino acids in their diets which they cannot themselves synthesize but which can be synthesized by the microorganisms of the rumen. Second, non-ruminants cannot handle efficiently foodstuffs like grass which are high in indigestible cellulose and other fibrous material, whereas the microorganisms of the rumen can digest cellulose. There is a third, less often emphasized ability of the ruminants, the ability to destroy certain toxic substances which harm non-ruminants. For instance, the gossypol of cottonseed is injurious to non-ruminants but not to ruminants.

Not only can ruminants synthesize body protein from protein, such as corn protein, which is deficient in some amino acids essential for non-ruminants, they can even synthesize body protein from non-protein materials such as urea and ammoniated carbohydrate, although these cannot be used as sole sources of nitrogen. (See Chapter 12.)

Thus discussion of some aspects of protein nutrition, such as amino acid composition, is directed mainly to the nutrition of non-ruminants.

Although, in general, a protein which is imperfect for non-ruminants is likely to be more satisfactory for ruminants which have microbiological aids to digestion and synthesis, there are instances of the opposite sort, in which a meal which is superior to another when tested with non-ruminants is actually inferior when tested with ruminants. (See Chapter 16.) Every feed, as has already been emphasized, must be evaluated under its actual conditions of use by a particular animal.

Amino acid composition. The value of a protein for non-ruminants depends, in the first place, on its amino acid composition. In general, animal protein foods, such as meat, milk, poultry, fish, and eggs, contain in adequate amounts all the essential amino acids which a non-ruminant needs for the synthesis of its own body protein. The vegetable protein foods for man in the American diet, mainly based on wheat, are, in contrast, deficient in some essential amino acids; almost all the lysine of the American diet comes from animal protein foods. The corn fed to swine and poultry is also deficient in certain amino acids and must in practice be supplemented by foodstuffs containing higher grade proteins. (See Chapters 2, 3, 8, 9, and 13.)

Plant vs. animal proteins. The National Research Council recommends that a man weighing 65 kilograms eat 65 grams of protein a day, and that a substantial portion be high-grade protein, which is taken to mean animal protein (1). (See Table I, Chapter 2.) It is a great mistake, however, which is emphasized by much of the contents of this book, to believe that good protein must *always* be animal protein. Poultry and swine, whose amino acid requirements are similar to those of man, can be raised entirely on protein from plant sources. (See Chapters 8, 14, and 17.) Half of mankind gets less, and many millions much less, than 15 grams of animal protein per capita per day. (See Appendix.) For the most part there is little immediate chance of great increase in the amount of animal protein which will be available, and hence the consumption of good vegetable protein is essential (1a).

Protein of metabolically active tissues. It is an important generalization that the proteins of actively metabolizing tissues, whether of plant or animal origin, all have about the same over-all amino acid composition, and all are, therefore, so far as composition is concerned, good proteins for the nutrition of non-ruminants. Thus the proteins of seed germ (such as wheat germ), of grass, and of microorganisms are about the same in over-all composition as the proteins of meat (2). There are disadvantages of the plant sources of metabolically active protein tissues,

but they are not disadvantages of amino acid composition. To illustrate the disadvantages, the protein of wheat germ is only a small part of the total wheat grain protein; the protein of grass is accompanied by cellulose, and even cellulose-free grass juice has an undesirable color and flavor; yeast is not an acceptable food when eaten by man in large amounts; and so on.

Structural protein. The animal proteins which have purely structural function, such as those in the keratin and collagen groups, usually are deficient in certain essential amino acids. But quantitatively they are a relatively small part of the animal protein that is eaten. In plants, the structural material is usually carbohydrate rather than protein in nature.

Storage protein. There is little storage protein in animals, with the major exception of the storage protein of eggs. In plants, however, storage protein is of the greatest importance, and most of this book is concerned with plant storage protein. The amino acid composition of plant storage protein is extremely variable in an unpredictable way. The plant, unlike non-ruminant animals, can synthesize all its amino acids from a variety of sources of nitrogen and so does not require that its storage protein contain all the amino acids. Some storage proteins, it so happens, like those of wheat and corn, are seriously deficient in certain amino acids essential for growth of non-ruminant animals, especially lysine. The proteins of some oilseeds, however, such as soybean and cottonseed, are, happily for non-ruminants, almost as complete in composition of amino acids needed to meet their nutritional requirements as meat protein. And other oilseed proteins, such as peanut protein, although less rich in lysine than soy or cottonseed protein, are still much better than wheat or corn protein. Furthermore, the proteins of many peas, beans, and pulses are usually of quite good quality. Details of amino acid compositions of different plant materials can be found in the appropriate chapters of Part II and are summarized in the last Chapter.

The very existence of some great civilizations of man and the development of some large livestock and poultry economies, such as the American swine and poultry economy, have depended on the basic fact that plant storage protein can be good protein. The technology of making utilizable the virtues of good plant protein is in great part the subject matter of this book.

Tests of biological value. The usual way of testing the nutritional value of a protein is to feed it to a young animal and to measure the rate of growth. To bring out any deficiency in the protein, the protein is usually fed at a relatively low level, and thus the diet puts a protein-

nutritional strain on the animal. Under this strain the animal easily shows the effect of any lack of some limiting amino acid. To give an important example, an animal fed on a low level of soy protein does not grow as rapidly as an animal fed on the same level of milk protein, unless the soy protein is supplemented with methionine, the limiting essential amino acid of soy protein.

In practice, however, it is not necessary to feed soybean protein at a low, "strain" level. Merely by feeding the soybean protein at a higher level, almost as good growth can be obtained with soybean protein as with milk protein. The exact level at which it is advisable to feed soy protein rather than accept a somewhat lower rate of growth or fortify the protein with methionine depends on a complex of nutritional and economic considerations which change with the state of knowledge and with the costs of the various materials. Always, it is the results under practical feeding conditions which are the ultimate criteria of the adequacy of a protein foodstuffs, not mere amino acid composition or "strain" feeding tests under laboratory conditions, however significant these may be for proper understanding of the mechanisms and practical possibilities. Of course, the actual feeding results, as will be discussed later in the book in more detail, must depend on the species and the age of the animal; rapidly growing animals, for instance, represent a sort of strain test for protein foodstuffs. Testing results must also depend on the composition of the entire diet. (See Chapter 7 for a discussion of methods of evaluation of protein quality.)

III. TECHNOLOGY

It has already been pointed out that the protein-nutritional value of processed vegetable protein foodstuffs cannot be considered adequately apart from the technology of the processing. The common animal protein foods—meat, milk, eggs, and fish—can be fed with little processing other than cooking. So can peas and beans. But the meals from oilseeds, for example, are commercial products, the residues of commodities which have been processed to obtain the valuable oil. They may have been processed further to remove harmful or undesirable ingredients, to make the meals stable, to supplement them with needed nutrients, and for many reasons that vary from product to product. Moreover, the oilseed meals which are suitable for adult animals often must be processed further to the point of isolation of the proteins to be suitable for man and for young pigs and calves. (See Chapters 10, 11, and 15.)

Different aspects of oilseed technology are in different historical stages. Removal of the oil is an ancient process, although this book will

discuss many modern technical improvements. (See Chapter 4.) Some of the oilseed meal technology is recent but already in widespread use, for instance, the proper toasting of soybean meal and the addition of vitamin B₁₂ of microbiological origin. Some of the modern technology, such as the making of cottonseed meal suitable for swine and poultry, is established on a moderate scale, but has yet to make its major impact. Finally, the use of isolated oilseed protein for man and for young mammals is, in its modern technological form, still in its infancy.

We shall try to summarize the general functions of the technology of plant protein foodstuffs, which will be illustrated in detail in the various chapters of this book.

Better oilseed meals. The techniques for removing oil from oilseeds were originally designed almost solely for maximum yield of oil at the lowest processing cost. Oilseed processing was called oil milling. Nowadays consideration is given to the quality of the oil as well as to its quantity, and new attention is given to the quality of the meal as a protein foodstuff.

A great impetus to the expansion of the soybean crop in the United States came from the cutting off of tropical oils during World War II. It is now obvious, however, that the soybean crop has not only supplied oil but has made possible the expansion of the American animal industry, particularly the poultry industry, by providing an increased supply of good protein. The money value of the meal is, indeed, now as great or greater than the value of the oil. It was very fortunate for the American animal industry that the soybeans originally raised for oil had so high a content of so high grade a protein. (See Chapter 14 on soybean oil meal.) As time goes on, it is likely that the relative importance of the protein parts of the oilseeds will increase.

A major improvement in soybean meal has been the controlled heating of the oil-free meal—enough heating to destroy antitrypsin and other harmful substances, but not enough heating to damage the protein. (See Chapters 5 and 14.) The present tendency is to improve meals further by increasing the percentage of nitrogenous matter; the mechanical separation and removal of indigestible fiber is being made more complete. Another improvement, whose potential significance is not yet widely recognized, is that the newer ways of removing residual solvent from a meal after the solvent extraction of the oil do not decrease the extractability of the protein, and yet do not involve any expensive removal of solvent under partial vacuum. (See Chapters 5 and 10.) Thus, as a first step in the preparation of isolated protein, it is now possible to extract practically all the protein from a meal which can be

produced at no extra cost, since there is no need for a special high-cost technique for removing solvent at low temperature.

The major advance in the processing of cottonseed meal has come from the realization that it is possible to inactivate the gossypol which is toxic to non-ruminants without causing much heat damage to the protein. Where the new knowledge has been put into practice, the value of cottonseed meal for feeding to poultry and swine has been increased. (See Chapter 17 on cottonseed meal.)

Quality evaluation of meals. The improvements in the processing of soybean and cottonseed meals, which have just been outlined and which are discussed in detail in the appropriate chapters, are given here as illustrations of the modern tendency to adjust the technology of producing oilseed meals to the increasing knowledge of the need to remove harmful substances and to avoid heat damage of the protein. (See Chapters 5 and 6.) The time has passed when the value of a protein feed is estimated only by weight or by nitrogen content. The educated buyer is now concerned with total nutritional value per unit of cost. Nutritionally inferior, unreliable, or variable meals must either disappear or be sold at inferior prices. This theme ties together much of the detailed technical discussion of the following chapters.

Supplementation of vegetable protein foodstuffs. Not only have oilseed meals been improved by changes in processing which remove or destroy undesirable substances and yet avoid heat damage to protein, but the value of the meals has in recent years been increased greatly by suitable supplements of small amounts of essential nutrients, especially of vitamins. Most of the early comparisons of the nutritive values of plant and animal proteins were distorted by the fact that the basic test diets did not contain all the needed vitamins and minerals. The animal protein often gave better results than the vegetable protein only because the animal protein diet was richer in vitamin impurities. It is now recognized that poultry, for instance, will grow as well on an essentially vegetable protein diet as on an animal protein diet, provided the vegetable protein diet contains suitable synthetic vitamins, microbiological products including antibiotics, and, at the most, a small amount of animal food containing minute amounts of unknown factors. All this supplementation can now be done economically.

If the animals of the United States had to get all their vitamin B₁₂ from fish meal and meat scraps, the only known practical sources a few years ago, the expansion of the animal industry and the proper utilization of the available bulk foodstuffs would have been seriously curtailed by the limited supply of these vitamin B₁₂-containing animal materials

(3). The advent of microbiological vitamin B₁₂ has had the immensely important effect of removing this limitation on the number of animals and the use of vegetable protein foodstuffs, and also has made the cost of vitamin B₁₂ low. Thus the processing of the protein foodstuffs from plant sources cannot be considered independently of the companion subject of vitamin requirements and the production of low-cost vitamins from non-animal sources.

Supplementation is by no means limited to the additions of vitamin or mineral concentrates. A particular feed, apart from its general value as a source of protein and carbohydrate, may itself be a rich supplementing source of a vitamin, as alfalfa is a rich source of provitamin A. (See Chapter 25.) A mixture of two meals, each alone inadequate in some one amino acid, may together have an adequate amino acid composition. (See Chapters 17 and 18.) And synthetic amino acids, such as methionine and lysine, can be used as supplements of defective proteins, just as synthetic vitamins can be added to plant materials lacking these vitamins. (See Chapter 13.)

Supplementation of plant protein foodstuffs with vitamins is only one item in the whole complex development of the production of animals for food. If the processed plant protein foodstuffs described in this book have been essential, to give one example, for the sensational rise in the American production of poultry meat along with a great lowering of its cost, so also have all the varied improvements in poultry raising aside from nutrition made possible the expanded use of these foodstuffs.

Isolated protein for man and young mammals. Although some unpredictable discovery may be just over the horizon, there are no radical changes in sight at present which are concerned with the utilization of protein meals for the usual animal feeds. The pattern of utilization is already established, and it will go on being extended and improved along the lines discussed throughout this book. In contrast, there is evidence for radical changes in the processing of plant protein for direct utilization by man and by young mammals. Such direct utilization is now insignificant in most of the western world and can become significant only if radical technological developments make the new uses possible. These developments, which are outlined in Chapters 10, 11, and 15, will probably consist in separating protein from the insoluble, indigestible carbohydrate and bad-tasting substances of oilseed meal, and in then giving the protein, which has been concentrated and made bland-tasting by the isolation process, such textural qualities and such nutritional and flavoring supplements as may be desired for each type of protein product.

IV. AVAILABILITY AND COST

The practical choice of a protein foodstuff for a farm animal or for man depends not only on considerations of amino acid composition and processing technology but also, obviously, on considerations of availability and cost.

Availability. To begin with, not every plant protein foodstuff can be produced everywhere. What is available depends on such factors as climate, soil, competitive demands on the land, and the state of agricultural, technological, and economic development.

Soybeans, so plentiful in the United States, are not raised and are not widely used in the main tropical areas of the world. Different tropical areas have either other protein-rich oilseeds, or sometimes no oilseeds of any significance as protein foods.

Western Europe has no large local supply of any oilseed meal suitable for animal feeding. It imports oilseeds, or oilseed meals, adjusting its imports to the shifting pattern of international trade. Although specific adjustments will vary from year to year, the general trend is toward reduction in trade in unprocessed materials. More and more of the great sources of vegetable oil and protein are processed in the countries where they are grown. (See the individual chapters in Part II for a detailed treatment.)

It is clearly not possible to survey here even in the most general way the problem of supply in the various parts of the world. But it must be emphasized that a realistic consideration of processed plant protein foodstuffs in any specific case must involve consideration of the local supply situation. Even within any one large country, such as the United States, it is economically advantageous to consume protein meals not too far from the region of production.

Cost. The problem of the costs of various processed plant protein foodstuffs for animals in relation to needs and financial resources and to the costs of alternative foodstuffs is so complex that it likewise cannot be even summarized here. The very concept of cost is itself complex. A high cost in labor may be preferable to a high cost in money or in land. A given cost in a local currency may be preferable to a lower cost in a foreign currency. A relatively high cost per unit of protein may be worth while if the high-priced protein material contains valuable non-protein ingredients needed for a particular diet. And whether the protein is the major product or a by-product of a particular plant will also be a factor. We must accordingly be content here to point out the crucial importance of the cost aspect of any protein feeding problem. Although there is no over-all discussion of protein feed costs in this

book, there are throughout the chapters many individual references to the economic side of protein raw materials and of their processing. A comparison of the costs for man of animal protein and isolated plant protein is given in Chapter 11.

V. THE FUTURE

The individual chapters of this book will discuss tendencies in individual fields. We should like to emphasize one broad tendency which has dominated and will continue to dominate the subject of plant protein foodstuffs as a whole: the ever-increasing demand by man for good-quality protein in ever more acceptable forms, a demand based on a constant increase of a world population doing less hard labor and having a rising standard of living.

The main result of this increasing demand for good and appealing protein foods must, of course, be an increasing demand for animal protein foods, and with it an increasing demand for processed plant protein foodstuffs for animal feeds. Even today, the demand for plant protein foodstuffs would be enormously greater than it is, were the best feeding practices universal. Were all the non-ruminant farm animals in the United States fed at what is now considered to be the optimum ratio of protein to carbohydrate, there would be a shortage of about 3 million tons of oilseed meals annually (4).

With more animals being raised and feeding practices becoming more sophisticated, there will be pressure to raise more protein foodstuffs over wider areas and to use them more efficiently. In North America, soybeans are now being raised in Louisiana and Canada as well as in the classical corn region around Illinois. Cottonseed meal is now being improved for use by non-ruminants. The ruminants, no doubt, will be fed on forages ever more effectively and will be using more urea and the like; thus protein meals of high quality will be spared for the non-ruminants.

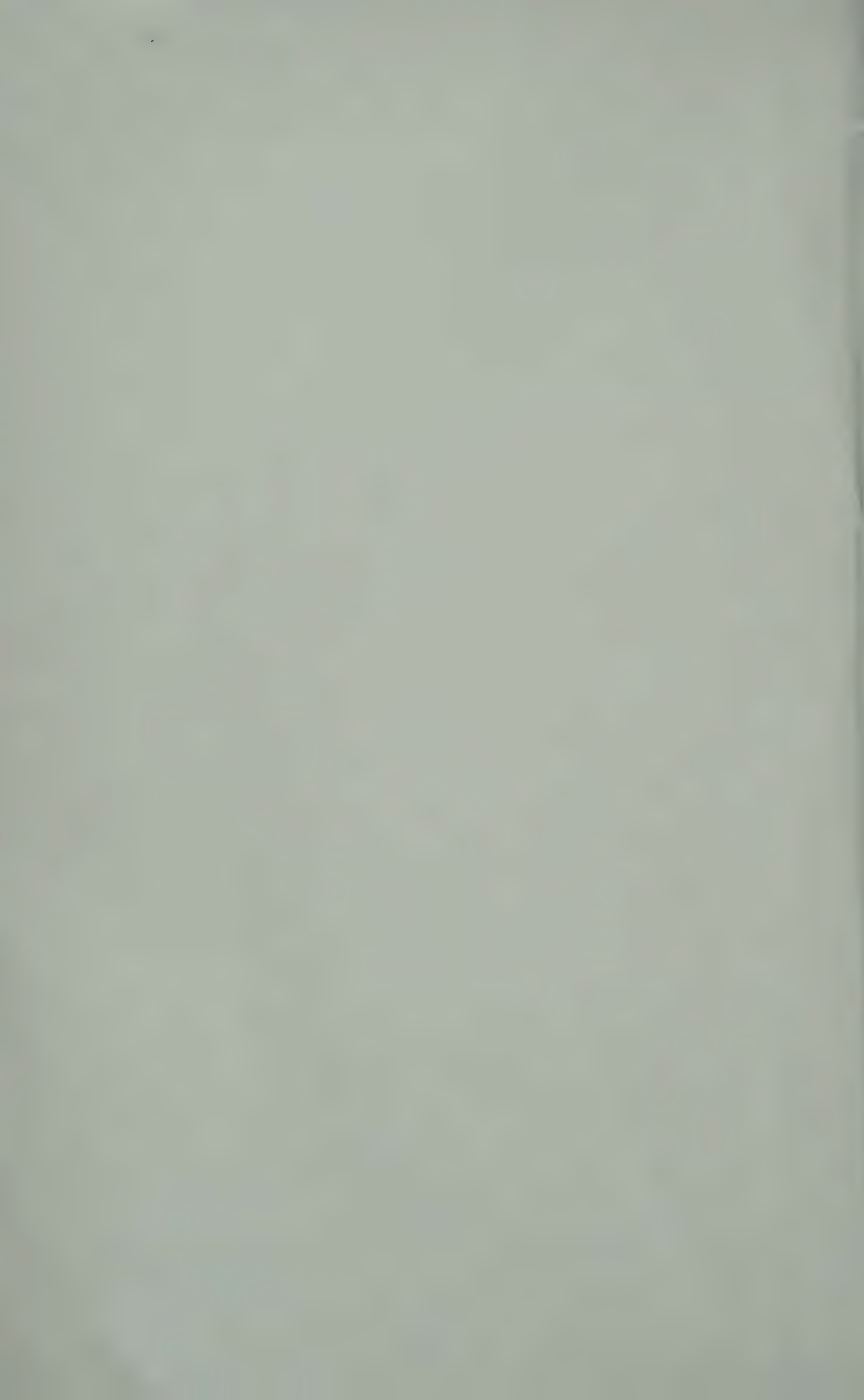
In some measure, the demand for attractive foods containing good-quality protein will be met by new foods based on the direct use of isolated oilseed protein. There are countries where an adequate expansion of the raising of animals is out of the question. In these countries, except where there is an adequate supply of cheap fish, good protein must largely be good vegetable protein. And even in those countries with an abundance of animal protein, the relative low cost of foods based on the direct use of vegetable protein will be a great force stimulating the development of vegetable protein foods.

Thus, constantly forcing change on the subject matter of this book are the prodigious pressures both of an increasing population and of a

basic change in man's diet in the direction of making it richer in good protein foods.

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PART I

**GENERAL PROPERTIES OF PLANT PROTEINS,
AND THEIR UTILIZATION**

CHAPTER 2

PROTEINS

C. M. LYMAN

This chapter is not addressed to the specialized protein chemist. Rather the author has attempted to present to those readers who perhaps may not have an extensive background in chemistry, but nevertheless have a vital interest in the use of protein products, a concept of what proteins are, including those facts about proteins which are of particular significance to him. With this objective in mind the first sections of the discussion have been translated from the language and expressions of the professional chemist to the language of every-day events.

A second and distinct purpose of this chapter is to serve as a source of references for technical information about proteins.

For modern developments in the chemistry and biology of proteins and amino acids, the reader is referred to the two-volume series, "The Proteins," edited by Hans Neurath and Kenneth Bailey (1), and to "Advances in Protein Chemistry," a yearly volume edited by M. L. Anson, Kenneth Bailey, and J. T. Edsall (2). Recent authoritative general texts include "Amino Acids and Proteins" by David M. Greenberg (3) and "Chemistry and Biology of Proteins" by Felix Haurowitz (4). A general discussion of vegetable proteins is in Chapter 3 that follows.

New developments concerning protein structure are described by H. D. Springall in "The Structural Chemistry of Proteins" (5).

I. PROTEINS, WHAT THEY ARE AND HOW ONE PROTEIN DIFFERS FROM ANOTHER

No form of life as we know it can exist without proteins. Even the simplest structures which have the capacity of reproducing themselves, such as certain viruses, invariably are constructed partially of proteins. What proteins are and what they do is thus of primary importance to us if we are to understand living organisms. Proteins are among the most important of food constituents.

What are proteins? Various definitions might be given, depending on which aspect of proteins we are considering and also on how complete we want our definition to be. For example, protein is the material which makes up the major part of the solid matter of muscle tissue and various organs of the body such as heart, kidney, and lung. This statement is true, yet proteins frequently make up a large portion of the kernels of oilseeds, and such materials appear to be quite different from a piece of meat.

To the organic chemist, proteins are complex organic compounds which invariably contain carbon, hydrogen, oxygen, and nitrogen and not infrequently other elements such as phosphorous. If we consider the matter in more detail we find that proteins are made up of structural units which can be separated one from another by chemical means and identified. Further we find that these structural units are not all alike. In fact more than twenty-two different kinds have been found in natural proteins from various sources. These structural units of proteins are called *amino acids*.

We may compare a protein molecule to a masonry building constructed of blocks of various kinds, sizes, and shapes. What the completed structure is like will be determined in part by the relative number of each of the different kinds of blocks used. The arrangement of the blocks is also a major factor in determining the characteristics of the building. Just as the masonry blocks are cemented together with mortar, the structural units of proteins are firmly bound together by chemical bonds.

How many proteins are there? When we consider that there are more than twenty-two different kinds of amino acids, that the relative amount of each amino acid in different proteins varies, and finally that the arrangement or sequence is of importance in the individuality of any protein, then we realize that an almost infinite number of different proteins are possible. Hundreds of them have been studied.

II. WHAT PROTEINS DO IN LIVING ORGANISMS

Structure. In animals the structural material of muscle and various organs consists primarily of proteins. The contractile material which serves in the final step of the conversion of the chemical energy of food to mechanical energy in muscular work is essentially protein.

Enzymes. Important as the structural function of protein is, proteins do far more than this. We may compare a living organism such as a human being or an animal to an extremely complicated factory with machines humming away at numerous locations, each machine doing its own particular job. In the living organism the vital part of

each specialized machine is an *enzyme*, and enzymes are specialized kinds of proteins. In some instances they are proteins combined with other chemical substances.

In a typical factory a whole series of machines is required to make a single finished product. The raw material is fashioned step by step as it passes from one machine to another. In living tissue whole series of specific enzymes function one after another in factory-like production line systems for the preparation of materials for building and repairing body tissue and for the production of energy from foods.

Hormones. Such a complicated organization must necessarily have a system for coordinating and controlling the many interrelated processes. In addition to the nervous system, a mechanism of chemical control operates through the discharge of "chemical messengers" into the blood stream by the endocrine glands. These chemical messengers or control substances are called hormones. Many hormones, but not all of them, are proteins.

In the plant world, the structural material is much more likely to be carbohydrate than protein. The mechanism of metabolic transformations in plants, however, as in animals, consists of coordinated enzyme systems. Plant enzymes as well as animal enzymes are specialized proteins.

In plant seeds, deposits of proteins serve as stock piles of stored food for the nourishment of the developing embryo.

III. THE SIGNIFICANCE OF PROTEINS IN ANIMAL AND HUMAN NUTRITION

1. Sources of Amino Acids for Tissue Formation and Repair

The formation of body tissue in a growing organism requires a never-ending supply of amino acids. In addition to the requirements for building new tissue, there is a constant wear and tear on existing body proteins, and these must be repaired with new amino acid units. Where do these building blocks come from? A number of kinds can be made within the tissues of the animal, that is, in muscle tissue, and in the liver, heart, and other organs. The starting materials may be food constituents such as sugar, starch, minerals, or sometimes fat.

It is well known that sugar and starch are important sources of energy for living organisms. As energy is produced, the sugar or starch is used up and carbon dioxide and water are formed. A simple illustration is the burning of coal in a steam engine operating to give mechanical power. The primary products of the fire are water and carbon dioxide. In living tissue, sugar is not burned directly to carbon dioxide

and water but is utilized through a complex series of chemical changes which finally result in the formation of these two products. During the process many organic compounds are formed, and some of these are suitable material for making certain types of amino acids.

Other kinds of amino acids cannot be made in the animal body but must be supplied in the food. These latter are called *indispensable* or *essential* amino acids. We may picture a building under construction. Certain types of structural units can be readily fabricated, shaped, and cut to size, right on the job. Other structural units must be shipped in exactly as they are to be used. If any of these latter materials fail to arrive when needed, the whole project comes to a halt. This is exactly what happens in tissue building when any one of the indispensable amino acids is left out of the diet. If the supply is not shut off entirely but only part of what is needed arrives on the job, then tissue building proceeds but at a slower rate. What about substitution of materials? As a general rule, in protein formation this does not occur. When one essential amino acid is missing, no protein is formed at all. Some protein chemists believe that a few of the amino acids which are quite similar in chemical structure are substituted one for another to a limited extent. If in certain instances this does happen, it is the exception rather than the rule.

In a typical food material, the amino acids are present in the form of proteins; they are not present to any significant extent as free amino acids. Before food protein can be utilized for tissue building, the amino acid structural units must be freed from each other. We have noted above that the chemical forces which hold them together in the complete protein are strong. Special machinery is required to take them apart without destroying them. This machinery consists of enzymes in the digestive tract which are called *proteolytic enzymes*, and the process by which proteins are taken apart is called *hydrolysis*.

Not all proteins can be hydrolyzed with equal ease. One reason for this is found in the way in which the amino acid structural units have been put together. Sometimes excessive heat during food processing converts the proteins into a form which the enzymes have difficulty in handling. When this happens food proteins are only partially digested. Digestibility is then one of the factors which must be taken into consideration in connection with the nutritional value of any given protein food material.

2. Protein and Amino Acid Requirements

We have seen that protein is required in the diet of animals and human beings for several different purposes. For maintenance, new

protein is constantly required to replace and repair existing body tissue; during growth, protein is required to build new tissue; and additional supplies are required for the production of milk during lactation and for reproduction. It follows, then, that the nutritional requirement for protein varies with the age and stage or development of the individual.

In considering the significance of protein requirements, it should be kept in mind that the values rest on the assumption that the protein to be used is of average nutritional value. Although proteins are all different and in the strictest sense, there is no average protein, most people in those areas where there is an adequate supply of protein eat quite a variety of foods, and consequently the nutritional value of the protein which they eat is of average value. When the diet or ration consists of only a few different kinds of foods or feeds, the quality of the protein must be given consideration (see discussion of amino acid requirements below). In spite of this limitation, tables of protein requirements are still useful guides in nutritional problems.

Protein requirements can be expressed in a number of different ways. We can say that an individual of such and such a size or weight requires so much protein per day. If we know how much food the individual will consume in a day, then the protein requirement can be expressed as a per cent of the total diet. For farm animals such as chicks and growing, fattening pigs, which are allowed to consume all the food they wish, the latter way of expressing protein requirement is much more useful. Since in reference tables the values are frequently given as per cent of crude protein in the diet, an explanation of the term crude protein seems in order.

Crude protein. Perhaps the most accurate way of determining the protein content of a food material would be to separate out all the protein by chemical means and weigh it. To make such a procedure really accurate requires a great deal of work even for a single food material. It happens that a high percentage but not all the nitrogen in foods is contained in the protein. The practical analytical procedure is, therefore, to determine total nitrogen, and on the assumption that an average protein contains 16% nitrogen, the total nitrogen content is multiplied by the factor 6.25 to give the protein content of the material. Obviously, a value for protein content obtained in this way includes small amounts of nitrogenous material which is not true protein. If nitrogenous material is present which cannot be digested by the animal, this is also included. The term crude protein is frequently applied to protein values obtained as indicated above.

Not all proteins contain exactly 16% nitrogen, and therefore the factor 6.25 is admittedly an approximation. For example, the more

accurate factor for wheat protein is 5.83. (See Chapter 33.) In attempting to use the more accurate values for individual feed ingredients, complications soon arise in connection with mixed feeds. Further complications arise owing to the lack of information on the more accurate factors for many materials. All in all, the most satisfactory practice seems to be to use consistently the factor 6.25 for the calculation of crude protein. This is the factor that is used throughout the book.

Protein requirements. Cattle and sheep normally consume large amounts of feeds which are high in fiber. Often the indigestible fiber

TABLE I
RECOMMENDED DAILY PROTEIN ALLOWANCES FOR HUMAN BEINGS^a

Group	Age (years)	Weight (lb.)	Protein required per day (g.)
Men	25-65	143	65
Women	25-65	121	55
Women, pregnant (third trimester)			80
Women, lactating			100
Infants	Birth to 1 year		Weight in kg. × 3.5
Children	1-3	27	40
	4-6	40	50
	7-9	59	60
	10-12	78	70
Boys	13-15	108	85
	16-20	139	100
	10-12	79	70
Girls	13-15	108	80
	16-20	120	75

^a Natl. Research Council Natl. Acad. Sci. (U.S.) Publ. No. 302, 22, rev. (1953).

carries with it substantial amounts of protein which also is not digested by the animals. For this reason protein requirements for cattle and sheep are expressed as digestible protein.

The large volume of literature which is available on protein requirements has been reviewed by committees of the National Research Council, and recommended protein allowances have been formulated. These recommendations have been summarized in Tables I (6) and II (7-12). (See also the discussion of protein requirements of human beings in Chapter 9 and of animals in Chapter 13.)

For farm animals, the recommended allowances represent good production practice. They do not necessarily mean that the amount recommended will give the maximum in growth rate. The protein in a farm feed is generally one of the more expensive constituents, and the law of diminishing returns sometimes makes it more profitable to

the producer to give slightly less than the amount required for maximum growth rate.

The policy in formulating recommended allowances for human beings has been quite different, and in this instance the values include a safe margin above the minimum requirement.

The nutritional requirement of an animal for protein is in reality a composite requirement for a number of different indispensable amino acids. Thus if we feed an experimental rat a 20% protein diet in which all the protein is supplied by gelatin, the animal will die because of the lack of the amino acids tryptophan and lysine.

Amino acid requirements. As is done for protein requirements, amino acid requirements can be expressed either as a requirement for so much per day or as per cent of the total diet. A third and perhaps the most useful way is to express the requirement for amino acid as per cent of the crude protein. This last method has some definite advantages in the evaluation of the protein in different kinds of food materials, such as those discussed in this book. For example, the protein in peanut meal contains 3.5% lysine, and the requirement of the chick for lysine is 4.5% of the protein. This means that peanut meal is deficient in lysine when used as a source of protein in chick rations. On the other hand, the protein in soybean meal contains 6.17% lysine, which is more than that required by the chick. (See chapters of Part II for detailed discussions of the individual proteins.)

The quantitative amino acid requirements of several species are given in Table III (13-41).

Tables showing the amino acid content of vegetable protein feeds are included in the last chapter. If it is desired to determine whether any given feed is adequate with respect to a particular amino acid, the amino acid content of the feed (expressed as per cent of protein) is compared to the requirement (also expressed as per cent of protein) listed in Table III. It will be noted that the quantitative requirements vary with different species.

The requirement for the amino acid methionine is complicated by the capacity of the animal body to convert methionine into cystine so that methionine can satisfy the need for both methionine and cystine. If the diet contains plenty of cystine, then the methionine requirement represents the requirement for this amino acid only. When cystine is absent or is present in suboptimum amounts, then additional methionine must be supplied to provide for cystine synthesis.

Similarly phenylalanine can be converted to tyrosine in the body, and additional phenylalanine is required if tyrosine is not present in the diet in adequate amounts.

TABLE II
RECOMMENDED PROTEIN ALLOWANCES FOR FARM ANIMALS^{a-f}
POULTRY^a

Chickens	Description of chickens: Crude protein, % of ration	0-8 wk.	8-18 wk.	Breeding hens	Laying hens
		20	16	15	15
Turkeys	Description of turkeys: Crude protein, % of ration	Starting poults 0-8 wk.	Growing turkeys 8-16 wk.	Breeding turkeys	
		28	20	15	
Ducks	Description of ducks: Crude protein, % of ration	Starting or growing ducks			
		17			
SWINE ^b					
Pigs, market stock	Weight, lb.: Crude protein, % of ration	25	50	150	250
		18	16	13	12
Pigs, breeding stock	Description of pigs: Crude protein, % of ration	Pregnant females and breeding boars		Lactating females	
		Young stock	Adult	Young stock	Adult
		15	14	15	14
DAIRY CATTLE ^c					
Normal growth of dairy heifers	Weight, lb.: Digestible protein, lb./day	50	100	200	400
		0.20	0.40	0.50	0.60
Maintenance of mature cows	Weight, lb.: Digestible protein, lb./day	800	1000	1200	1600
		0.50	0.60	0.70	0.80
				600	800
				0.85	0.90
				1000	1200
				0.95	1.00

Reproduction Add to maintenance during last 2 to 3 months, digestible protein, lb./day 0.60

Lactation		Fat content of milk					
		Add to maintenance for each pound of milk,					
		digestible protein, lb./day					
		3.0%	4.0%	5.0%	6.0%		
		0.040	0.045	0.050	0.055		

Maintenance of	Weight, lb.:	1200	1600	2000	2400
breeding bulls	Digestible protein, lb./day	1.00	1.20	1.45	1.60

BEEF CATTLE ^d									
Heifers and steers, normal growth	Weight, lb.:	400	600	800	1000				
	Digestible protein, % of ration	7.5	5.6	4.7	4.3				
Bulls, growth and maintenance	Weight, lb.:	600	800	1000	1200	1400	1600	1800	
	Digestible protein, % of ration	8.1	8.2	7.0	6.5	5.8	5.4	5.4	

Wintering weanling calves	Weight, lb.:	400	500	600					
	Digestible protein, % of ration	6.4	6.2	5.3					

Wintering yearling cattle	Weight, lb.:	600	700	800	900				
	Digestible protein, % of ration	5.0	4.7	4.5	4.5				

Wintering pregnant heifers and mature cows	Digestible protein, % of ration	4.5							
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Cows nursing calves, first 3 to 4 months after parturition	Digestible protein, % of ration	5.0							
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TABLE II (Continued)

Fattening calves finished as short yearlings	Weight, lb.:	400	500	600	700	800	900
	Digestible protein, % of ration	9.2	8.6	8.1	7.8	7.5	7.2
Fattening yearling cattle	Weight, lb.:	600	700	800	900	1000	1100
	Digestible protein, % of ration	7.2	7.0	6.8	6.7	6.5	6.3
Fattening two-year-old cattle	Digestible protein, % of ration	6.3					
SHEEP ^e							
Bred ewes	Digestible protein, % in ration	5.0					
Ewes in lactation	Weight, lb.:	100	110	120	130	140	150
	Digestible protein, % in ration	6.0	6.0	6.0	6.2	6.1	6.1
Ewes—lambs and yearlings	Weight, lb.:	70	90	110	130		
	Digestible protein, % in ration	7.3	6.9	5.7	5.3		
Rams—lambs and yearlings	Weight, lb.:	75	100	125	150	175	
	Digestible protein, % in ration	6.8	6.0	6.0	5.3	5.1	
Fattening lambs	Weight, lb.:	50	60	70	80	90	
	Digestible protein, % in ration	8.1	7.8	7.0	6.9	6.7	
HORSES ^f							
(Values expressed as daily allowances)							
Growing horses	Mature weight	200	400	600			
	600 lb.	0.77	0.57	0.45			

Mature weight 800 lb.	Weight, lb.:	200	400	600	800
	Digestible protein required, lb.	0.93	0.80	0.65	0.54
Mature weight 1000 lb.	Weight, lb.:	200	400	600	800
	Digestible protein required, lb.	1.04	0.94	0.77	0.64
Mature horses	Weight, lb.:	400	600	800	1000
	Digestible protein required:				
	Maintenance, lb.	0.31	0.42	0.53	0.62
	Light work, lb.	0.38	0.52	0.65	0.76
	Medium work, lb.	0.46	0.62	0.77	0.91
	Heavy work, lb.	0.52	0.70	0.87	1.03
Mares—last quarter of pregnancy	Weight, lb.:	400	600	800	1000
	Digestible protein required, lb.	0.46	0.62	0.77	0.91
Mares—lactating	Weight, lb.:	400	600	800	1000
	Digestible protein required, lb.	1.01	1.37	1.70	2.01

^a Poultry: *Natl. Research Council Natl. Acad. Sci. (U.S.) Publ. No. 301 (1954)*.
^b Swine: *Natl. Research Council Natl. Acad. Sci. (U.S.) Publ. No. 295 (1953)*.
^c Dairy cattle: "Recommended Nutrient Allowances for Dairy Cattle," *Natl. Research Council Natl. Acad. Sci. (U.S.) Rept. No. 3, rev. (1950)*.
^d Beef cattle: "Recommended Nutrient Allowances for Beef Cattle," *Natl. Research Council Natl. Acad. Sci. (U.S.) Rept. No. 4, rev. (1950)*.
^e Sheep: "Recommended Nutrient Allowances for Sheep," *Natl. Research Council Natl. Acad. Sci. (U.S.) Rept. No. 5, rev. (1949)*.
^f Horses: "Recommended Nutrient Allowances for Horses," *Natl. Research Council Natl. Acad. Sci. (U.S.) Rept. No. 6 (1949)*.

Ruminants. The amino acid requirements of cattle, sheep, and other ruminating animals are quite different from the requirements of monogastric animals. In fact, the amino acid composition of protein feeds for cattle and sheep has little, if any, influence on the growth and development of the animals. The reason is that a ruminating animal is supplied with a large storage tank called a rumen. The feed which the animal eats goes directly to this storage tank, and there countless millions of microorganisms live which have the capacity of synthesizing

TABLE III
QUANTITATIVE AMINO ACID REQUIREMENTS

Amino acid	Adult human ^a (g./day)	Refer- ence ^b	Rats ^c (% of diet)	Chicks ^d (% of crude protein)	Refer- ence	Young pigs (% of crude protein)	Refer- ence
Arginine	0.0	16	0.2	6.0	24		
Histidine	0.0	17	0.4	0.75	25, 26	1.54	34
Isoleucine	0.70	18	0.5	3.0	27	3.2	35
Leucine	1.10	18	0.8	7.0	27	4.6	34
Lysine	0.80	19	1.0	4.5	28, 29	5.5	36
Methionine	1.10 ^e	20	0.5	2.25 ^g	30, 31	2.0 ^g	37
Phenylalanine	1.10 ^f	21	0.7	4.5 ^h	32	3.6 ^h	38
Threonine	0.50	20	0.5	2.25	33	3.0	39
Tryptophan	0.25	22	0.2	1.0	30	0.75	40
Valine	0.80	23	0.7	4.0	27	3.1	41

^a The recommended safe daily intake is twice the minimal amount listed in the table.
^b General reference is: W. C. Rose, *Federation Proc.* **8**, 546 (1949).
^c References are: W. C. Rose, *Physiol. Revs.* **18**, 109 (1938); M. Sahyun, "Proteins and Amino Acids in Nutrition." Reinhold, New York, 1948.
^d Recalculated from original literature on the basis of 20% protein diets.
^e Includes quota for synthesis of cystine.
^f Includes quota for synthesis of tyrosine.
^g Additional methionine is required if the diet is low in cystine.
^h Additional phenylalanine is required if the diet is low in tyrosine.

the amino acids which the animals cannot make themselves. These small forms of life (bacteria, etc.) tear apart the protein which the animal eats and rebuild it to form their own bodies. Later the microorganisms die and are, in turn, digested by enzyme secretions discharged into the digestive system by the animal. The net result is that the relative amounts of the amino acids which reach the blood stream are determined not by the food that the animal eats but by the amino acid composition of the microorganisms. It happens that the amino acid distribution pattern in the bodies of the microorganisms is satisfactory for supplying the needs of the animal.

Rumen microorganisms not only have the ability to refashion food proteins, but they also have the capacity of making new protein out of simpler nitrogenous compounds such as urea. The use of urea in cattle and sheep feeds is discussed in Chapter 12.

3. Protein Metabolism in Disease and Injury

The above discussion of protein and amino acid requirements is based on the needs of normal healthy individuals. During disease, and also after burns, surgery, fractures, or other injury, the amount of protein required for rapid recovery is usually higher than the amount needed by a comparable healthy individual. At the same time the tendency is for a person who is ill to decrease food intake. In an excellent discussion of the problem, Pollack and Halpern (42) point out that inadequate protein nutrition during disease can result in (1) delay in convalescence, (2) poor wound healing, (3) liver injury, and (4) increased susceptibility to infection and lowered resistance to disease.

A brief consideration of some of the major factors involved is given below. It has been demonstrated by Schoenheimer, Rittenberg, and associates (43–45) that body tissues are in a state of dynamic equilibrium. Hence body tissues are not static materials which exist in the body until they are worn out and then discarded. Rather they are constantly being broken down and rebuilt. During the process there is a certain wastage of amino acids, and the nitrogen from this waste is excreted in the urine and feces. The body's mechanism for tissue repair during disease and after injury involves an acceleration of this whole process. Consequently there is an increased excretion of nitrogen in the urine, and the amount of good-quality protein needed in the diet is increased.

Another important concept in connection with this problem is that there are no extensive protein reserves in the body. It is true that in time of stress the body can draw from blood protein and that the liver can supply proteins to other parts of the body with resulting decrease in size of the liver. If this process goes too far, then the liver becomes susceptible to damage by toxic agents which may arise from bacterial infection or other sources.

One of the most important body mechanisms in combatting infection is the production of antibodies in the blood stream. These antibodies remove the toxins from the blood and destroy the invading organism as well. Antibodies are proteins, and hence their continued production depends on an adequate supply of amino acids. Cannon and his co-workers (46–48) have demonstrated that severe restriction

of the dietary protein intake in an otherwise adequate diet results in a reduced capacity to produce antibodies of several kinds.

Dieting of individuals who have a tendency toward obesity is undoubtedly important for health and longevity. A growing recognition of the importance of adequate protein nutrition is the reason that reducing diets now recommended by the medical profession are frequently high-protein diets.

4. Methods for Determining the Nutritional Value of Proteins

Long before reliable quantitative data on the amino acid composition of proteins were available, the nutritional value of the proteins in a variety of foods was studied by direct feeding trials. Several distinctly different types of experimental procedures have been used. Since an evaluation of these methods is given in Chapter 7, only a brief statement is included here.

One of the simplest procedures is to feed the protein to be tested as the sole source of protein in an experimental diet which is otherwise nutritionally complete. As a general rule the protein should be fed at a level less than the amount which will produce optimum growth. Young, growing rats frequently are used as experimental animals. When several proteins are compared, the relative increase in weight of the animals fed the different proteins over a given time gives a relative measure of the nutritional value of the protein.

In a modification of this procedure, the increase in weight per gram of protein eaten is used as the unit of measurement. Investigators are divided in their opinion as to whether the animals should be allowed to eat all they want or whether the food intake should be equalized (paired feeding trials).

In a second general type of procedure developed in detail by Cannon and associates (49), experimental animals are depleted of body protein by keeping them for a period of time on a protein-free diet or on one very low in protein. During this period the animals lose about 30% of their body weight. Equal weights of the protein to be tested are then fed daily, and the increase in weight of the animals per unit of protein is recorded. Values are expressed as protein efficiency.

There are only two major factors which determine whether one protein is nutritionally superior or inferior to another. These are (1) its digestibility and (2) the suitability of its amino acid distribution pattern for building and repairing body tissue. Each of the two types of procedures for evaluating proteins mentioned above give answers which represent over-all values.

A third type of procedure based on nitrogen balance measurements provides for the evaluation of these two factors separately. Digestibility can be determined by measuring nitrogen in the feed and in the feces. Then if the amino acids which are absorbed and reach the blood stream are in the proper proportions, a young, growing animal will use them for building body tissue. If the proportions are not correct, then a considerable proportion of the amino acids will be used for energy and the nitrogen will be excreted in the urine. Pioneering work in this field was done by Thomas (50), and the procedures

were refined and extensively applied to food materials by Mitchell (51). According to Mitchell the biological value of a protein is defined as "that percentage of the absorbed nitrogen which can be utilized for maintenance and growth." Thus the term, biological value, has a very specific meaning, whereas the term, nutritional value, has a more general connotation.

The Thomas-Mitchell method takes into consideration the fact that, even in the absence of any protein in the diet, some nitrogen is excreted as a result of normal metabolic processes. The method also takes into consideration the fact that not all the nitrogen excreted in the feces represents undigested food material, but some of it comes from digestive enzymes secreted into the digestive tract and also bacteria which live in the lower sections of the intestine. The formula for the calculation of biological value is then

Biological value =

$$\frac{\text{N intake} - (\text{fecal N} - \text{metabolic N}) - (\text{urinary N} - \text{endogenous N})}{\text{N intake} - (\text{fecal N} - \text{metabolic N})} \times 100$$

In this formula metabolic N is the nitrogen which appears in the feces and endogenous N is the nitrogen which appears in the urine during a control period when the diet is practically free of protein.

TABLE IV
THE DIGESTIBILITY AND BIOLOGICAL VALUE
OF THE PROTEIN IN DIFFERENT FOODS^a

Food	Digestibility (corrected)	Biological value
	%	%
Whole egg, cooked	100	94
Milk	100	85
Egg white, cooked	100	83
Beef liver	90	77
Beef round	96	69
Pork, ham	100	74
Rolled oats	90	65
Whole wheat	91	67
White flour	100	52
Whole corn	95	60
Potato	78	67
Navy beans, cooked	76	38
Cocoa	38	37
Chocolate	38	37

^a Values reported by H. H. Mitchell and G. G. Carman, *J. Biol. Chem.* **68**, 183 (1926)

The digestibility and biological values of the proteins in some foods as reported by Mitchell (52) are given in Table IV. Particular attention is called to the wide variation which occurs in the biological value of different proteins. Similar variations in the protein quality of food products can be demonstrated by protein repletion tests or by growth studies.

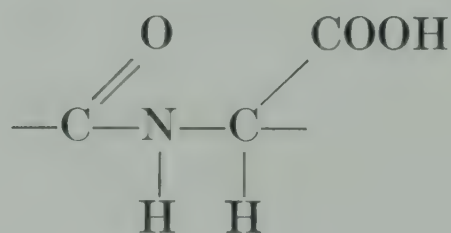
It must be kept in mind that when two proteins are fed together an amino acid deficiency in one protein may be counterbalanced by the other protein.

In such instances the experimentally determined biological value of the mixed proteins will be higher than the value for either protein alone. Biological values for the protein in mixed feeds therefore cannot be calculated as an average of the proteins contained in the feed. For example, Mitchell and Kick (53), using young pigs, determined the biological value of corn, tankage, and a mixture of the two. The values obtained were 54%, 42%, and 61%, respectively. In experiments with young rats, Mitchell and Carman (54) found that the biological value of white flour was 52% and for whole egg the value was 94%. The calculated value for a mixture of two parts flour and one part egg would be 66%. The experimental value for the mixture was 75%.

Although the determination of biological value is fundamentally sound, the procedure is very laborious. Perhaps a more serious disadvantage is that, although supplemental values of different proteins can be demonstrated, there is no way of predicting such supplementation without a knowledge of the amino acid composition of the products. This same limitation applies to any other type of protein evaluation method which is based on animal feeding trials.

IV. STRUCTURE AND CHEMICAL PROPERTIES OF PROTEIN

The primary chemical bond in a typical protein is the peptide linkage which binds two amino acids together through the amino group of one and the carboxyl group of the other. This structure may be represented as



The chemical force involved is strong; separation by chemical reagents requires drastic treatment (heating with 4 *N* NaOH or 6 *N* HCl). Although these linkages cannot be separated by boiling with water or heating in an oven at similar temperatures, they can be readily separated by the proteolytic enzymes at room temperature.

The evidence is now overwhelming that in a protein the amino acids are formed into long chains held together by peptide bonds. The evidence is also convincing that these long peptide chains are coiled or folded into definite configurations which are important factors in the chemical and biological individuality of a protein. These folded or coiled configurations are maintained primarily by secondary and much weaker forces such as hydrogen bonds and salt linkages.

Thus, a specialized protein like an enzyme loses all its biological activity on boiling with water. Its solubility and physical properties are also drastically altered, although quantitative analysis for the individual amino acids shows that the same amounts are present as in the

original protein. Essentially all that has been changed is the disruption of the orderly arrangement of the peptide chains brought about primarily by breaking the hydrogen bonds and salt linkages.

When a protein has lost its characteristic arrangement of the peptide chains we say that it is *denatured*. Proteins vary considerably in the ease with which they are denatured. For example, some enzymes cannot be handled in the laboratory at room temperatures without losing their activity. In such instances experiments must be conducted at near-freezing temperatures.

The nutritional significance of these facts about protein structure is that the appearance and physical properties of a protein can frequently be changed considerably by heat or other treatment without altering the nutritional value. Nutritional value is damaged only when amino acids are actually destroyed or when arrangements or reactions are caused to take place in the peptide chain which make the proteins resistant to the action of the digestive enzymes.

One important addition needs to be added to our concept of protein molecules as long chains of amino acids coiled or folded into specific forms which may have the external shape of spheres, ovals, or rods. Many of the amino acids have characteristic chemical groups, and these groups extend out from the core of the structure of a protein. Essentially the reactions of protein are the reactions of these groups. By way of example, the amino acid lysine has an amino group on the end of the molecule (ϵ or epsilon amino group). When proteins are heated with carbohydrates, this amino group reacts with the carbohydrate to form a protein-carbohydrate complex. It happens that amino groups on the outside of a protein molecule are necessary for the rapid action of the digestive enzymes which take the protein apart, so that the amino acids can be absorbed from the digestive tract. When the amino groups are blocked by reaction with carbohydrate, protein digestion is impaired (55). (For further discussion of this important relationship, see Chapter 5.)

Perhaps the above brief discussion may seem to oversimplify the problem of protein structure. Actually, little more than a solid beginning is known today about this important subject, and the field is wide open for fundamental discoveries. One of the recent important contributions has been the development of methods for determining the sequence of amino acids along the carbon chains. Using this approach the complete arrangement of the amino acids in the peptide chains of insulin has been worked out by Sanger and co-workers (56, 57).

Among numerous proposals concerning the coiling or other arrangements of the chains the models presented by Pauling and Corey (58)

have received the widest general acceptance and seem to be in accord with X-ray and other experimental data. (For general references on protein structure, see 1, 2, 5.)

V. PROTEIN CLASSIFICATION

It would appear that the total number of different proteins which occur in various forms of life is almost unlimited. A satisfactory classification of proteins according to type and distinctive characteristics would therefore be very useful. All proteins contain carbon, hydrogen, and nitrogen, and a great many contain sulfur. Classification on the basis of elemental composition is accordingly not practical. Attempts have been made to classify proteins on the basis of their amino acid composition. There are some striking differences, but the individuality of a protein is dependent on the arrangement of the amino acids as well as on the total amount of each contained in the protein. Further, there are many distinctly different proteins which have a somewhat similar amino acid distribution. For these reasons classification on the basis of amino acid composition has not been generally satisfactory.

Some years ago no general discussion of proteins would have been considered complete without the classification based on solubility which was recommended by the Committee on Protein Nomenclature (59) in 1908. During recent years it has become apparent that a single protein could be classified in two or more ways, depending on the procedure used in the preparation. Also, so many proteins have been found that seem to be in between two types that the solubility classification is today given less and less attention. Nevertheless, group names like albumin, globulin, and glutelin still appear in the current literature, and for this reason the classification is given below. (See Chapter 3 for a discussion of classification as applied specifically to vegetable proteins.)

The Classification of the Proteins

1. Simple proteins yield on hydrolysis only amino acids or their derivatives.
 - a. *Albumins* are soluble in water, coagulable by heat, and usually deficient in glycine.
 - b. *Globulins* are insoluble in water, soluble in strong acids and alkalies, and in neutral salts, and usually contain glycine.
 - c. *Prolamins* are soluble in 70 to 80% ethyl alcohol, yield large amounts of proline and amide nitrogen, and are deficient in lysine. They have been isolated principally from cereal seeds.
 - d. *Glutelins* are heterogeneous mixtures of cell proteins obtained by alkaline extraction of the residues after removal of the albumins, globulins, and prolamins.
 - e. *Scleroproteins* (albuminoids) are all those fibrous proteins which have a supporting or protective function in the animal organism. In the plant

kingdom they are probably represented by cellulose and similar substances.

- (1) *Collagens*, the principal supporting proteins of skin, tendons, and bones, are resistant to peptic and tryptic digestion and are converted into an easily digested soluble protein, gelatin, by boiling with water.
 - (2) *Elastins*, present in elastic tissues such as tendons and arteries, are digested by trypsin, are not converted into gelatin, and give a negative color test for hydroxyproline.
 - (3) *Keratins* are proteins resistant to digestion by pepsin and trypsin, insoluble in dilute acids and alkalies, in water, and in organic solvents, and, on acid hydrolysis, yield such quantities of histidine, lysine, and arginine that the molecular ratio of these amino acids, respectively, is approximately 1:4:12.
- f. *Histones* are soluble in water, are precipitated by dilute ammonia, and contain large amounts of the basic amino acids, especially lysine and histidine. They are usually found in animal tissue, united as salts, with acidic substances such as heme and nucleic acids.
- (1) Globins are basic proteins (histones) which contain tryptophan, tyrosine, arginine, histidine, and lysine in a molecular ratio of approximately 2:3:3:8:9.
- g. *Protamins* are basic polypeptides first found in ripe fish spermatozoa; they can be subdivided into four groups, depending on their content of the basic amino acids:
- (1) Arginine only (monoprotamins)
 - (2) Arginine and lysine (diprotamins)
 - (3) Arginine and histidine (diprotamins)
 - (4) Arginine, histidine, and lysine (triprotamins)

Protamins usually exist in combination with nucleic acids.

2. Conjugated proteins are proteins united with some substance which on decomposition does not yield amino acids only. The common conjugated proteins include (a) nucleoproteins, (b) glycoproteins, (c) phosphoproteins, (d) chromoproteins, and (e) lipoproteins.

VI. PROTEIN ANALYSIS

1. Qualitative Tests

Several qualitative color tests for proteins are based on the chemical reactivity of the side chains of certain amino acids.

a. Millon's Reaction

When a protein is heated with a solution of mercuric nitrate and mercuric nitrite in a mixture of nitric and nitrous acids, a red color or precipitate is obtained. The reaction is due to the phenolic side chain of tyrosine.

b. Xanthoproteic Reaction

On heating a protein with concentrated nitric acid, a yellow color which changes to orange on treatment with base is obtained. The color is due to the nitration of the benzene ring of the amino acids tyrosine, tryptophan, and phenylalanine.

c. The Biuret Test

This test differs from the other two in that the color reaction does not depend on the presence of any particular amino acids but rather on peptide linkages which are present in all proteins. In this test a violet color is obtained when a protein is mixed with sodium hydroxide solution and a very weak solution of copper sulfate.

Details of these and additional color tests may be found in Hawk *et al.* (60).

2. Quantitative Determination

For the determination of the protein content of foods, farm feeds, and other agricultural products, the only practical general procedure is to determine total nitrogen (Kjeldahl method) and to calculate the protein content by the use of an appropriate conversion factor. For details of the experimental procedure, see "Official Methods of Analysis of the Association of Official Agricultural Chemists" (61). The conversion factor of 6.25, which is based on the assumption that a typical protein contains 16% nitrogen, is generally used. The significance of the approximations and limitations involved in such a procedure have been discussed in a previous section.

In biochemical investigations it is frequently necessary to distinguish non-protein nitrogen from protein nitrogen. The most general procedure is to effect a separation by precipitation of the protein.

For the determination of soluble proteins such as, for example, blood proteins, good colorimetric procedures are available (62, 63). The intensity of the color obtained with different proteins varies, and hence a pure sample of the protein to be studied should be used as a standard.

VII. AMINO ACIDS

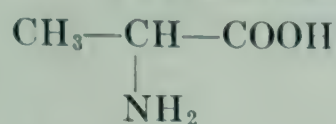
1. Chemical Properties

Highly purified samples of amino acids appear as white crystalline

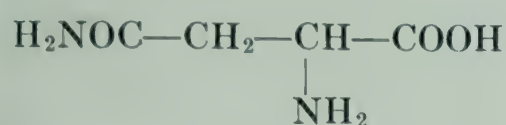
substances. Chemically, they all have free carboxyl groups ($\text{—}\overset{\text{O}}{\underset{\text{||}}{\text{C}}}\text{—OH}$), and all except proline and hydroxyproline have free amino groups (—NH_2). The chemical reactions which characterize amino acids as a class of compounds are the reactions of these two chemical groups. In addition, the different kinds of amino acids all have different side chains, some of which are quite reactive. The reactions which distinguish one amino acid from another are due to these side chains. The chemical structures of the amino acids which occur in natural proteins and some which do not ordinarily occur in proteins but do occur in tissues as free amino acids or in other forms are given in Table V.

TABLE V

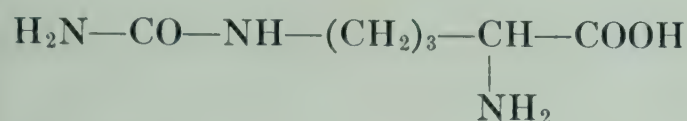
THE CHEMICAL STRUCTURE OF THE NATURALLY OCCURRING AMINO ACIDS



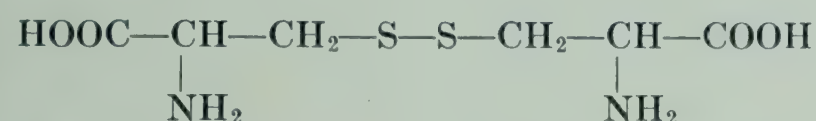
Alanine



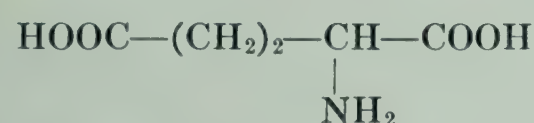
Asparagine



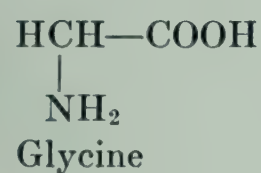
Citrulline



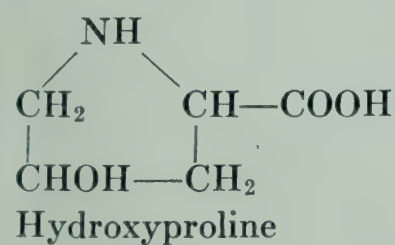
Cystine



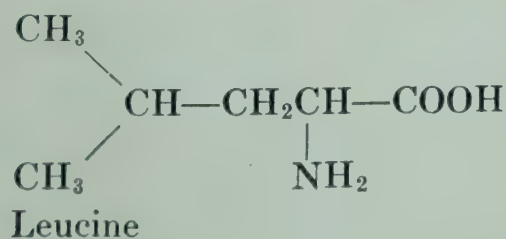
Glutamic acid



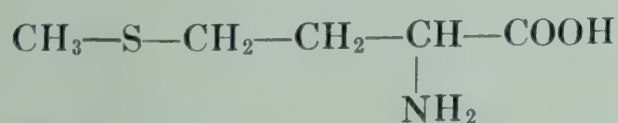
Glycine



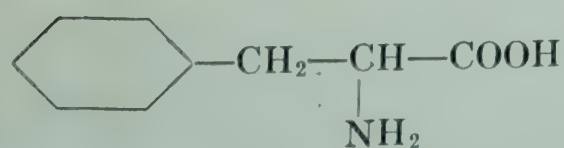
Hydroxyproline



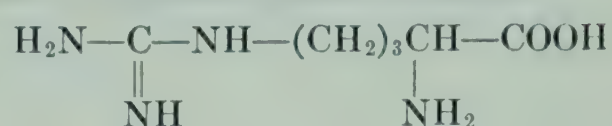
Leucine



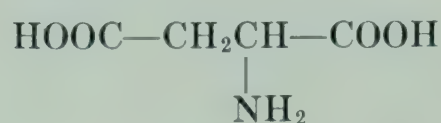
Methionine



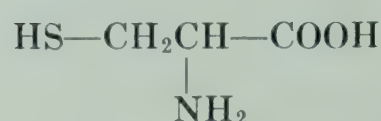
Phenylalanine



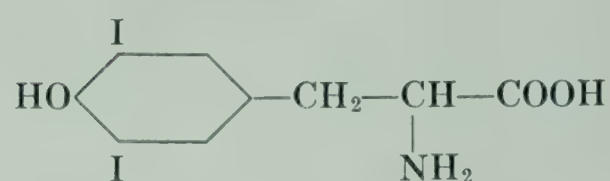
Arginine



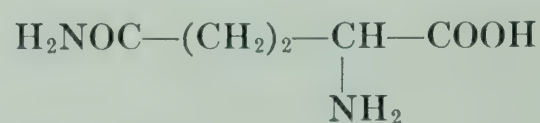
Aspartic acid



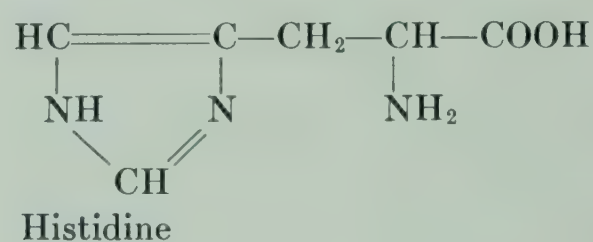
Cysteine



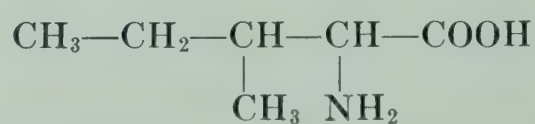
Diiodotyrosine



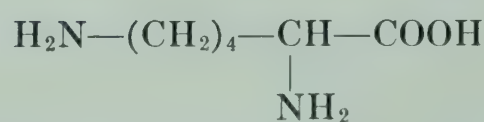
Glutamine



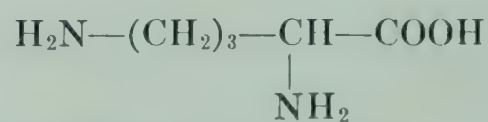
Histidine



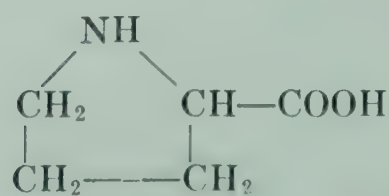
Isoleucine



Lysine

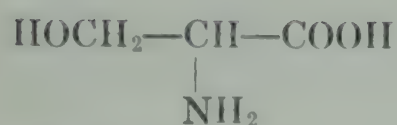


Ornithine

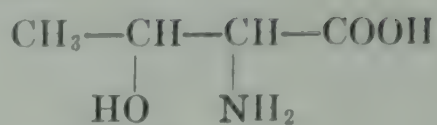


Proline

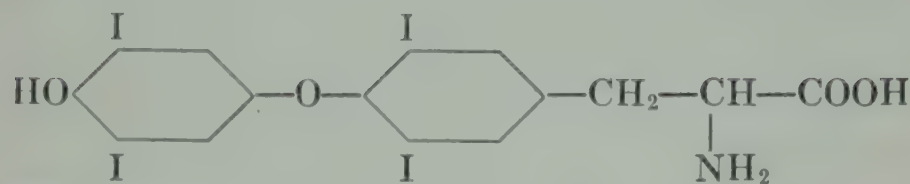
TABLE V (Continued)



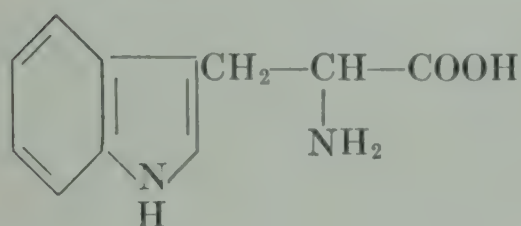
Serine



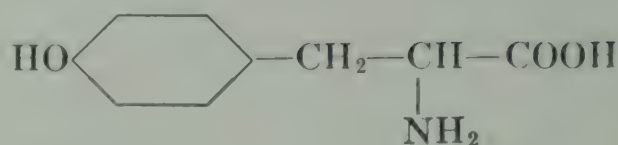
Threonine



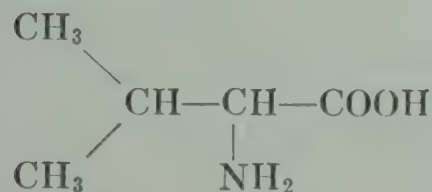
Thyroxine



Tryptophan



Tyrosine



Valine

It is far beyond the scope and purpose of this book to present the detailed chemistry of the amino acids. For further information, the reader is referred to general biochemistry textbooks such as West and Todd (64), Harrow and Mazer (65), Haurowitz (66), and Fruton and Simmonds (67), and to specialized books on proteins and amino acids such as Greenburg (3), Neurath and Bailey (1), and Haurowitz (4).

2. Analytical Methods

a. Hydrolysis of Proteins and Foodstuffs

Except in certain colorimetric procedures, the first step in the determination of the amino acid composition of proteins and foods is the hydrolysis of the material to liberate the amino acids. Three general methods have been used: (1) acid hydrolysis, (2) basic hydrolysis, and (3) enzymatic digestion. For acid hydrolysis, constant boiling hydrochloric acid (approximately 6 *N*) has proved to be most generally useful. For basic hydrolysis, 4 to 5 *N* sodium hydroxide is usual. For enzymatic digestion, mixtures of proteolytic enzymes, generally pepsin and trypsin, have been recommended.

Each procedure has its limitations. In the presence of carbohydrates, tryptophan, tyrosine, and cystine are completely or partially destroyed during acid hydrolysis. Racemization occurs during basic hydrolysis,

and this must be taken into consideration in microbiological assays. Tyrosine and cystine are partially destroyed during basic hydrolysis. Although complete liberation of amino acids from foodstuffs has been accomplished with proteolytic enzymes, each new product to be analyzed presents a separate problem, and evidence concerning the completeness of hydrolysis is needed. Some peptide residues are extremely difficult to hydrolyze completely with enzyme mixtures. This limitation is so severe that enzymatic hydrolysis is seldom used for the preparation of samples for amino acid determinations.

b. Determination of Amino Acids

Methods for the quantitative determination of amino acids may be divided into several groups, depending on the general type of procedure. Some advantages and disadvantages of the general methods and some selected references are given below.

(1) *Quantitative isolation.* Historically one of the first methods used to determine the amount of a given amino acid in a protein was to separate out chemically the amino acid and weigh it. In later modifications of these methods, the amino acids were converted to more insoluble derivatives which were easier to separate. The problem is to purify the precipitate without a significant loss of material. In the determination of the amino acid content of food materials containing carbohydrates and other non-protein materials, these difficulties are of such a magnitude that quantitative isolation procedures are seldom used today.

Arginine is precipitated as the diflavianate and recrystallized as the mono-flavianate (Vickery, 68). This is a good method.

Many papers have appeared on the determination of histidine as the silver salt. The review of these methods, by Block and Bolling (69), indicates that the values obtained by these methods are likely to be low.

Methods for the determination of lysine based on the isolation of the picrate are of doubtful significance.

Methods for the determination of glutamic and aspartic acids have been based on the insolubility of the barium or calcium salts in alcoholic solution. Bailey *et al.* (70) reviewed these methods and concluded that as generally applied the procedures were far from quantitative.

The precipitation of tryptophan as the mercury salt has been used as the basis for analytical methods (69, 71).

(2) *Isotopic dilution methods.* As a class of analytical methods, isotopic dilution ranks high in accuracy and reliability. These methods have not been extensively applied to food materials because of the requirements for materials, equipment, and specialized skill. In order to

determine a given amino acid, a supply of this amino acid labeled with an isotopic element, usually nitrogen or carbon, must be available. A known amount of a labeled amino acid is added to the unknown hydrolyzate. The amino acid, or a derivative of it, is then isolated and purified. Quantitative recovery is not required, since the amount of the amino acid in the unknown mixture is determined by the dilution of the isotopic label in the isolated amino acid.

The equation used in the calculation is

$$B = (C_0/C - 1)A$$

where B = the amount of amino acid originally present.

A = the amount of labeled amino acid added.

C_0 = the concentration of the labeled element in the added amino acid.

C = the concentration of the labeled element in the isolated amino acid.

In a modification of the isotopic dilution method the amino acid in the protein hydrolyzate is labeled by causing it to react with *p*-iodophenylsulfonyl chloride (called pipsyl chloride for convenience) in which the iodine or sulfur is radioactive (72, 73). Amino acids which have been determined by the isotopic dilution method include arginine (74), lysine (74–76), glutamic acid (75–78), tyrosine (74–76), glycine, and alanine (72).

(3) *Color reactions.* Colorimetric methods for the determination of amino acids in proteins have been used for many years. Some of these have stood the test of repeated reinvestigation, whereas many others have been shown to be lacking in reliability, usually with respect to specificity. Hydrolyzates from food materials containing carbohydrates are usually dark in color, and this frequently presents a difficulty and sometimes a source of error.

Histidine is determined colorimetrically after reaction with diazotized sulfanilic acid (Pauly reaction). The modification of Macpherson (79, 80) is one of the most satisfactory. Because tyrosine as well as histidine reacts with diazotized reagents, a preliminary separation of the two is usually necessary.

Several colorimetric methods are available for the determination of tryptophan. One of the most reliable of these is based on the reaction with *p*-dimethylamino-benzaldehyde and sodium nitrite in strong acid solution (81–84). The method can be applied to intact proteins (85).

Tyrosine is determined by the Millon reaction (reaction with mercury salts and nitrite in acid solution to give a red color). Many modifications of the procedure have been successfully used (86–90).

In the Kapeller-Adler (91) method, phenylalanine is nitrated with potassium nitrate in concentrated sulfuric acid followed by color development with hydroxylamine in ammoniacal solution. The method has been modified by Block and Bolling (69).

Methionine is determined colorimetrically by the McCarthy and Sullivan (92) reaction. The color is developed by reaction with nitroprusside in strongly alkaline solution.

(4) *Microbiological assay*. Microbiological assays have proved to be one of the most valuable general types of analytical procedure for the determination of the amino acid composition of foods and farm feeds. Some of the advantages of these methods are as follows: (1) It is not necessary to separate out the protein before hydrolysis, since carbohydrate and other decomposition products do not interfere with the assays. (2) The procedure is relatively simple and is applicable to large numbers of samples. (3) Only very small amounts of material are required. A sample containing 100 mg. of protein is sufficient to determine ten to fifteen different amino acids.

In principle, a microbiological assay is a feeding trial with a strain of some microorganism as the "experimental animals"; all nutrients except the amino acid to be determined are supplied in the media. Graded amounts of a standard amino acid solution and of unknown hydrolyzates are added to a series of tubes, and the growth of the organism over a given period of time then reflects the amount of the amino acid present in the unknown. Growth is determined by turbidity or, in the case of the lactic acid bacteria, by the amount of acid produced.

Cultures of the organisms can be obtained from the American Type Culture Collection, Washington, D. C. Catalogue numbers are as follows:

Leuconostoc mesenteroides P-60 (ATCC 8042), *Streptococcus faecalis* R (ATCC 9790, 8043), *Lactobacillus arabinosus* 17-5 (ATCC 8014), *Lactobacillus brevis* (ATCC 8257), *Lactobacillus citrovorum* (ATCC 8081), *Lactobacillus delbruekii* LD-5 (ATCC 9595), and *Lactobacillus casei* (ATCC 7469).

A number of the amino acids can be determined satisfactorily by the use of any one of several organisms. In other cases one particular organism gives decidedly more consistent and reliable results. The early work has been reviewed by Snell (93), Schweigert and Snell (94), and Dunn (95). The organisms which have given the best results in the author's laboratory, together with a limited number of references, are given in Table VI (96-118).

(5) *Chromatography*. During recent years a great many analytical methods have been developed based on either paper or column chromatography. For the application of these methods to amino acid analysis, reference is made to the books by Lederer and Lederer (119) and by Block *et al.* (120). To date, the application of these methods to the analysis of food materials for amino acid content has been limited.

TABLE VI
METHODS FOR THE MICROBIOLOGICAL DETERMINATION OF AMINO ACIDS

Amino acid	Preferred assay organism	Reference	Other satisfactory assay organisms	Reference
Alanine	<i>Leuconostoc citrovorum</i>	96		
Arginine	<i>Leuconostoc mesenteroides</i> P-60	97	<i>Streptococcus faecalis</i> R <i>Lactobacillus casei</i>	98-100 101
Aspartic acid	<i>Leuconostoc mesenteroides</i> P-60	102	<i>Lactobacillus delbrueckii</i>	103
Glutamic acid	<i>Lactobacillus arabinosus</i> 17-5	100, 102, 104	<i>Leuconostoc mesenteroides</i> P-60	100
Glycine	<i>Leuconostoc mesenteroides</i> P-60	99, 100		
Histidine	<i>Streptococcus faecalis</i> R	98, 105	<i>Leuconostoc mesenteroides</i> P-60	99, 106, 107
Isoleucine	<i>Lactobacillus arabinosus</i> 17-5	97, 108	<i>Streptococcus faecalis</i> R <i>Leuconostoc mesenteroides</i> P-60	98 99
Leucine	<i>Lactobacillus arabinosus</i> 17-5	97, 99, 108	<i>Streptococcus faecalis</i> R	98
Lysine	<i>Leuconostoc mesenteroides</i> P-60	97, 99, 106, 109	<i>Streptococcus faecalis</i> R	98
Methionine	<i>Leuconostoc mesenteroides</i> P-60	97, 100, 110	<i>Streptococcus faecalis</i> R	98, 99
Phenylalanine	<i>Leuconostoc mesenteroides</i> P-60	97, 111	<i>Streptococcus faecalis</i> R	98
Proline	<i>Lactobacillus brevis</i>	112	<i>Leuconostoc mesenteroides</i> P-60	100
Threonine	<i>Streptococcus faecalis</i> R	97, 98, 105, 113		
Tryptophan	<i>Lactobacillus arabinosus</i> 17-5	97, 99, 114, 115	<i>Streptococcus faecalis</i> R	98
Tyrosine	<i>Leuconostoc mesenteroides</i> P-60	99, 117, 118	<i>Lactobacillus casei</i>	116
Valine	<i>Lactobacillus arabinosus</i> 17-5	97, 99, 108	<i>Streptococcus faecalis</i> R	98

Moore and Stein (121) have described in full detail a procedure for the analysis of amino acid mixtures based on the use of a column packed with the ion exchange resin Dowex 50. This development marks an important step forward in protein analysis. It is anticipated that further developments will be made in this field and that chromatography will be used more extensively in the future for amino acid analysis of foodstuffs.

(6) *Miscellaneous*. Methods have been developed for the determination of a number of amino acids by decarboxylation with specific enzymes and measurement of the carbon dioxide evolved. With the proper preparation of the specific enzyme these methods can be made quite accurate and reliable. Procedures have been described for arginine (122), histidine (123), lysine (123, 124), and glutamic acid (123). Application to the analysis of food materials has not been very extensive.

Other methods are based on the oxidative degradation of the amino acid molecule. For these and other miscellaneous methods, see Olcott (125).

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CHAPTER 3

PLANT PROTEINS

A. BONDI

I. INTRODUCTION

Plant proteins are, of course, the subject matter of the entire book. Although there is a voluminous scientific and review literature on the subject of plant proteins, this book would not be a complete entity if it did not present, in an introductory manner, an over-all view. Many of the subjects discussed in a general way in this chapter will be presented in later chapters in greater detail. It is our thought that this general presentation should aid the reader as he follows the detailed treatments later on. [*Editor*]

Proteins are important constituents of all plants, being the seat of and directing the course of all their manifold activities, as well as having structural and storage functions. The characteristic chemical composition and structure of these proteins, which are so important metabolically in plant development, determine their value as nutrients for animals. (See the reviews in the literature (1-7a).) Their fundamental properties were first recognized by the three great nineteenth century chemists: Ritthausen, Schulze, and Osborne (1, 2).

Ritthausen pointed to the importance of amino acid analyses for the characterization of plant proteins, but only the development of exact analytical techniques in recent years made correct amino acid analyses possible. Such analytical data were compiled by Block and Bolling (8), Tristram (8a), and others, and are reported throughout this book.

Schulze investigated the metabolic phenomena that occur when plant seeds sprout and develop into seedlings; his most remarkable observation concerns the disappearance of reserve proteins of the seed and the enrichment of the seedling in amides such as asparagine. The correctness of his statements was confirmed many years later by using tracer techniques.

Osborne did the outstanding work on the isolation of seed proteins. For this purpose the seeds were extracted with dispersing solutions from

which the proteins were precipitated by salting out. The modern methods of isolating proteins for technical or scientific purposes are based on these same fundamentals.

A great number of plant proteins are known. The chemical and physical properties of the different proteins are not characteristic enough in most instances to permit a suitable classification. For instance, a classification based on the solubility of proteins is rendered difficult by the fact that they occur in nature almost invariably in complex mixtures and thus, by interaction, modify each other's solubility. A classification of vegetable proteins according to their location in plant tissues, their biochemical function, or more precise physicochemical properties would be the more advisable. Nevertheless, the use of solubility properties as a basis for differentiation of the protein fractions is universal because of the relative ease of the manipulations. Most plant proteins are categorized by this method.

In this chapter plant proteins are divided into two groups: the reserve proteins of seeds, and the functional proteins of the vegetative parts of the plant (leaves, stalks, and roots). Attention is not confined to proteins alone but is given also to simpler nitrogen compounds such as peptides, amino acids, and amides. These compounds were neglected by most of the former workers but now are recognized as important intermediate products formed during the synthesis of plant proteins, and their value as nutrients for rumen bacteria seems to be established.

II. SEED PROTEINS

1. General Discussion

The seeds are the raw material for some of the most important food-stuffs for human and animal nutrition, and their different components have, therefore, been investigated extensively. They contain all the food material required by the seed embryo for its initial development—protein, starch, fat, essential growth factors, and a moderate amount of minerals. The embryo becomes the rapidly developing part on germination, and hence it contains a large number of enzymes and considerable amounts of proteins appropriate to the functions of development. When exposed to proper conditions of moisture and temperature, the enzyme systems become fully active and the phenomena of growth are initiated. Hydrolysis of the seed proteins takes place during germination, and the resulting amino acids become the raw materials for the formation of cell proteins for the growing seedling.

The higher flowering plants are divided botanically into two main groups according to the structure of the seed. The dicotyledons, e.g.

legume seeds, produce seeds that more or less readily split into two halves, or cotyledons, with the embryo between them. The monocotyledons, the group including cereal grains, bear seeds with only a single storage organ usually called the endosperm, which is characterized by its content of a considerable amount of starch.

a. Preparation

Isolation of pure plant proteins encounters several difficulties. As pointed out previously, these substances occur in plants as complex mixtures of several proteins together with other constituents of the tissue in various stages of association or conjugation. Methods for the preparation of pure proteins without changing their natural properties require mild treatments inasmuch as proteins are denatured (lose their original natural properties such as solubility) even before any changes in their chemical properties can be observed.

For preparation of pure seed proteins, the fat is usually first removed from the dehulled seeds by extraction with organic solvents at relatively low temperatures, a procedure essential in order to avoid physical and chemical changes. Water-soluble, non-protein nitrogenous substances can be removed by ethanolic aqueous extractants (5), and starch by use of 21% hydrochloric acid (9).

Protein is extracted from defatted seeds by a dispersing agent, which may be water, a salt, alkaline or acid solution, or some organic solvent. The insoluble residue and protein dispersion are separated by mechanical means; the protein is precipitated by heat, dialysis, electrodialysis, salts, acids, bases, or organic chemicals. Choice of the precipitating agent depends on the agent originally used for peptizing the protein. An ideal peptizing agent should bring all the proteins into true solution or dispersion without changing the original structure of the protein. By use of drastic methods such as extraction with strong acid and alkali, protein can be dissolved quantitatively or almost quantitatively, but its original structure is then changed (10). Many processes, for example the use of dilute alkali, effect the simultaneous solution of different protein fractions. Therefore, reproducible results are obtained only by working under rigorously controlled conditions. (See also Chapter 10.)

b. Classification

Seed proteins can be separated for purposes of crude classification into four different fractions by successive use of the following solvents: water, salt solutions, 70% ethanol, and dilute alkali (or acid). Albumins are soluble in water, globulins in salt solutions, prolamins in 70% alcohol, and glutelins in alkali (11). (See also Chapter 2.) The most im-

portant properties of these four fractions of seed proteins are the following:

1. *Albumins* are soluble in water. This is the outstanding characteristic which distinguishes them from other proteins. They are also soluble in dilute neutral salt solutions and are precipitated only by high concentrations of salts such as saturated ammonium sulfate solution. Albumin solutions are stable over a considerable range of acidity or alkalinity. They are characterized by insolubility in organic solvents; hence they may be precipitated by ethyl alcohol. Albumins are at least minor components of all plant cells, and small amounts are also found among the reserve proteins of most seeds, as is the leucosin of cereal seeds. In spite of considerable differences in their amino acid compositions, the albumins have as common property a small glycine content.

2. *The globulins*, which (unlike the albumins) are insoluble in water, are soluble in dilute, neutral salt solutions. They are precipitated by moderate salt concentrations, frequently by half-saturated ammonium sulfate. The globulins show a slightly higher isoelectric point than albumins, indicating a slightly higher basicity (pH 5.5 to 6.5 instead of 4.5 to 5.5 as for albumins). In contrast to the albumins, the globulins usually contain considerable amounts of glycine. The nitrogen percentage of globulins is comparatively high (18 to 19% N content) owing to the high content of diamino acids, particularly arginine. The percentage of glutamic acid is likewise high, and, therefore, the globulins exhibit no basic properties. Most of the chemical properties of albumins and globulins, on the other hand, are very similar, and it is sometimes difficult to distinguish one from the other. The globulins, like the albumins, are insoluble in organic solvents and are precipitated from solution by ethyl alcohol. They are found in the cells of all plants and are one of the characteristic types of proteins of seeds, particularly of seed embryos. Among the salt-soluble globulins, edestin from hemp seed has received considerable attention. Other similar proteins are glycinin from soybeans, excelsin from Brazil nuts, amandin from almonds, legumin from peas and lentils, phaseolin from beans, and conavalin and concanavalin from jack beans. Cottonseed and pumpkin seeds also contain globulins. Globulins are extracted from the ground seeds by 2 to 10% sodium chloride solutions, and many of them can, under suitable conditions, be obtained in crystalline form (6). The separation of crystals is no guarantee of homogeneity, but it suggests that the solid phase is a relatively simple mixture of closely allied compounds (12).

3. The ethanol-soluble *prolamins* contain a considerable percentage of the amino acid, proline, from which they take their name. Prolamins contain only small amounts of basic amino acids (lysine), but high amounts of glutamic acid. In spite of this high content of glutamic acid, the prolamins are not acidic proteins because the carboxyl groups of all the glutamic acid molecules exist as amides. Prolamins, such as gliadin from wheat or rye, hordein from barley, or zein from maize, are prepared by extraction from flour with 70 to 80% ethanol and precipitation by addition of diethyl ether. The prolamins of wheat and rye are soluble in cold alcohol (60 to 70%), whereas those of corn or sorghums are soluble only in hot alcohol. These proteins differ also in their amino acid composition. Gliadin (from wheat) and hordein (from barley) are rich in tryptophan and lysine. Both of these amino

acids are lacking in zein (from corn) and, therefore, zein is of lower nutritive value. The prolamins are not single pure proteins and can be separated by electrophoresis into two or more components (10).

4. The *glutelins*, like the prolamins, are a mixture of closely related individual proteins which constitute an important portion of the reserve protein of cereal seeds (glutenin from wheat, hordenin from rye, avenin from oats, zeinin from maize). They are insoluble in alcohol and neutral salt solutions, are soluble in dilute alkali, and are precipitated by neutralization with acid.

In addition to these four classes of simple proteins, the seeds also contain conjugated proteins which contain non-protein moieties as intrinsic parts of their molecule. There are also small amounts of nucleoproteins which are contained in chromosomes and plant viruses (13).

2. Cereal Proteins

a. Protein Content of Cereals

Grains contain only a comparatively small amount of protein, approximately 10%. Nevertheless, cereal proteins are very important from the nutritional point of view, because they form a great part of the daily food of men and farm animals, and hence they are included in this discussion even though materials from cereal grains form but a small part of the subject matter of this book. In addition, these cereal proteins are necessary for the formation of a spongy, elastic, and cohesive dough when flour is wetted with water. The presence of only a comparatively small amount of protein in the flour, therefore, makes the baking of bread possible (14, 14a).

The protein content and the distribution of the different protein fractions of cereal grains are presented in Table I.

A factor of 5.7 is generally used to convert nitrogen into protein content for wheat and wheat products, instead of the customary factor of 6.25; this factor (5.7) is based on the average nitrogen content of gluten which is 17.55%. The factor of 6.25 is used for conversion from nitrogen content to protein for other cereals and is based on the assumption that these proteins contain 16% nitrogen. There seems to be no valid evidence for this one factor, but it is a firmly established convention. According to Heathcote (15), the nitrogen content of oat protein is 18.5% (a conversion factor of 5.40). Factors suggested for use in converting percentages of nitrogen in various substances into terms of proteins are tabulated by Joslyn (16). (See also Chapter 33.)

Considerable fluctuations in protein content and composition of grains may result from varietal differences and environmental conditions (soil, rainfall, and location). Rapid maturation has been found to

favor the production of grains of higher protein content. In general, starchy kernels of lower protein content are obtained in regions with mild, humid climates where there is a slow maturation and a long growing season (17). Regions with hot, dry weather yield grain with a relatively high protein content. In seasons of high rainfall or by the use of irrigation, there is a general lowering of the protein content. Variations in available soil moisture are responsible for this result; therefore, moisture-retentive soils tend to yield grain of relatively low protein content. Furthermore, it was found that the nitrogen level in wheat

TABLE I
PROTEIN CONTENT OF THE CEREALS^a

Name	Protein, (% dry grain)	Protein fractions, (% total protein)			
		Albumins	Globulins	Prolamins	Glutelins
Common wheat	10-15	3-5	6-10	40-50	30-40
Durum wheat	12-18				
Rye	9-14	5-10	5-10	30-50	30-50
Barley	10-16	3-4	10-20	35-45	35-45
Oats	8-14	1	80	10-15	5
Rice	8-10	Trace	2-8	1-5	85-90
Corn (maize)	7-13	0	5-6	50-55	30-45
Sorghum				60	
Common millet	7-16		10-11	57	30
Italian millet	10-11		13-14	48	37

^a Adapted from S. Brohult and E. Sandegren, in "The Proteins" (H. Neurath and K. Bailey, eds.), Vol. 2, Part A, p. 493. Academic Press, New York, 1954.

(18) and oats (19, 20) is related to the level of available nitrogen in the soil; their protein content can be raised substantially by use of nitrogenous fertilizers. For instance, the nitrogen content of 127 samples of oat kernels of the same variety grown at different locations in England fluctuated between 1.72 and 3.44%; the mean value was 2.43% nitrogen (19).

Varieties of corn differ from each other substantially in their protein content, and significant increases in the percentage of crude protein through fertilization were reported by Flynn *et al.* (21). The range of protein in the low-protein corn was 8.8 to 10.3% (average 9.9%) and in the high-protein corn 12.8 to 15.9% (average 14.3%). Various parts of the cereal grain kernels contain different amounts of protein: wheat germ contains 30.9%, bran 16.7%, and the endosperm 14.25% protein.

Hence, the protein content of flours varies according to the percentage of extraction.

b. Types of Proteins

Glutelins and prolamins constitute the bulk of the proteins of most kinds of cereal grains; only rice and oats contain small amounts of prolamins. The proteins of oats contain about 80% of the globulin avenin, and rice protein contains more than 80% of the glutelin oryzenin. The greatest relative amount of prolamins is contained in corn where the prolamins zein accounts for over half of its protein content. Glutelins and prolamins are considered to be storage proteins and are contained in the endosperm cells. The albumins and globulins are not storage proteins. Their role and site are not very well defined; they are found partly in the embryo and partly in the endosperm.

It can be assumed that the different protein fractions of rye, rice, corn, and sorghum described by Osborne (1) are not homogeneous chemical compounds. Scallet (22) showed that the prolamins of corn, zein, is composed of several components which may be distinguished by their different electrophoretic mobilities. It was also observed that changes in the concentration of protein fractions occur in mature grains during storage (23).

c. Proteins of Wheat

The proteins of wheat have been studied extensively because of the practical importance of gluten (14, 14a). Gluten is composed of two fractions: glutenin, a glutelin soluble in dilute alkali, and gliadin, a prolamins soluble in aqueous alcohol. In addition, the wheat seed contains significant amounts of an albumin, leucosin, and of globulins.

The importance of wheat for baking purposes is due largely to the unique property of the proteins of the wheat endosperm in forming gluten when wetted with water. When wheat flour is mixed with water, the proteins hydrate, forming gluten, a typically ductile, tenacious, elastic, coherent mass. This property is responsible for the superiority of wheat over the other cereals for the manufacture of leavened products as it makes possible the formation of a dough which will retain the carbon dioxide produced by yeast or chemical leavening agents. The quantity and quality of the gluten determine to a large degree the use for which a wheat flour is most suitable. For the production of yeast-leavened bread, flours milled from hard wheats are preferred, and for pastries, soft flours are more suitable. Hard flours are characterized by a relatively high percentage of protein with elastic gluten properties. The gluten associated with soft flours is tender, non-elastic, and easily torn apart (24).

Almost pure gluten is obtained as a residue of the preparation of starch from wheat. This residue can be used as an adhesive, as food for diabetics, as a source of glutamic acid, and in animal feeds. The laboratory preparation of gluten is accomplished customarily by mixing flour and water in a dough mixer and separating the gluten on a screen. A laboratory method of preparing dried purified wheat gluten was developed by Lusena (25); it consists in dispersing washed gluten in very dilute acetic acid, centrifuging, and vac-ice drying the dispersion.

Glutenin and gliadin have long been considered to be individual proteins, but recent research has revealed that each of these consists of several fractions which may be distinguished by their different physical properties and by fractional precipitation with neutral salts (26, 27). The results obtained by use of ultracentrifugal, electrophoretic, and diffusion methods could by no means confirm Osborne's characterization of wheat gluten as a simple mixture of two individual proteins. Those dealing with gluten from a practical standpoint, however, retain the terms glutenin and gliadin as the more convenient, since the nature of the different components of gluten has as yet not been ascertained, and, therefore, a more adequate classification is lacking.

d. Proteins of Barley

The proteins of barley were thoroughly investigated in recent years because of their importance in the industrial "malting process" (28). Malting of barley is an artificially induced germination conducted in darkness at temperatures between 10° and 25°. During the first stages of malting, the prolamin fraction, hordein, and the glutelin are converted into salt-soluble fractions. The nitrogen compounds of barley play an important part in brewing, since they are essential for yeast growth and are thought to be directly concerned with the production of foam. A remarkable influence of degree of grinding of barley kernels on the distribution of nitrogen between the salt-soluble, alcohol-soluble, and insoluble protein fractions was found by Halverson *et al.* (29). According to these authors the proportion of the different protein fractions differs also with several varieties of barley. Changes taking place in the distribution of the different protein fractions during the ripening of barley grains were investigated by Bishop (30). Folkes and Yemm (31) report that the four chief protein fractions of barley grains have been separated and their amino acid contents determined. The albumin and globulin closely resemble the protoplasmic protein of barley seedlings, whereas hordein and hordenin differ widely from it.

e. Amino Acid Composition

Amino acid analyses of various cereal proteins are listed in other parts of this book. As a class, the cereal proteins do not have as high a biological value as do the proteins of legumes, oilseeds, and nuts, or animal proteins. Lysine deficiency is the salient lack in the cereal

grains; they are also low in tryptophan content. Differences found in the leucine, valine, and methionine content are of minor significance. Rice and oat proteins are superior to those of wheat and corn. Sure (32) states that the proteins in buckwheat are the best known source of plant proteins of high biological value; this surprising result, which is based on nutritional experiments, has not yet been checked by others. It may be pointed out that the germ proteins contain larger percentages of lysine and tryptophan than do the endosperm proteins. It appears from nutrition experiments that whole wheat proteins are superior to the white flour proteins, and these biological results agree with the amino acids analyses. Gluten is particularly rich in glutamic acid, which may occur in a proportion as high as 46% of the total in wheat gliadin. The glutenin fraction contains 27% glutamic acid, whereas in zein of corn, 36% of this amino acid has been found. Wheat gluten and corn protein are used as raw materials for the preparation of monosodium glutamate, which is added to meats, soups, and cooked vegetables to intensify their flavors.

Heat treatments used in the preparation of bread and of many breakfast foods (baking or toasting) result in some destruction of lysine and tryptophan in wheat and corn, of lysine in rice, and of cystine in oats. The loss of lysine in bread due to baking was found to be about 15% (33). This loss in biological value is attributed to the browning or Maillard reaction (34) between sugars and lysine at higher temperatures which results in its reduced availability (35). (See Chapter 5.)

The amino acid composition of cereal proteins is influenced by growth conditions and varieties, and sometimes the proportion of certain amino acids can be changed by altering the conditions under which the cereals are grown. Such changes were observed for wheat (36), oat (37), and corn (21) proteins.

The amino acid composition of cereal foods for human use is of primary importance if the food in question forms a large part of the diet, for example, rice in the Far East; otherwise, the amino acid deficiencies in cereal proteins may be compensated by the proteins of other foods. Similarly, cereals as suppliers of amino acids for monogastric farm animals (pigs and poultry) may be complemented by foods which are more efficient as sources of the limiting amino acids.

3. Dicotyledonous Seed Proteins

✓ Dicotyledonous seeds are much richer sources of proteins than are cereal seeds; hence, oilseeds are essential protein suppliers for man and animals. In 1952, 50 million tons of oilseeds containing 20 to 30% protein were harvested throughout the world; these oilseeds are used

primarily as a food, and only a small quantity is for industrial purposes. The larger-seeded legumes, such as dry beans, peas, and lentils, which contain 22 to 25% protein, are of secondary importance for the supply of proteins for man and animals.

The main and most characteristic fraction of proteins contained in oilseeds is the globulin fraction which generally constitutes 80% of the proteins. The other three fractions are not present or are present in small quantities. Protein can be isolated from most of the leguminous seeds relatively more easily than from cereals, by means of the principles outlined previously. For instance, soybean proteins for food purposes are extracted by use of only slightly alkaline or neutral salt solutions. The solubility of the seed proteins is influenced by materials such as potassium phosphate, lecithin, and other naturally-occurring materials in seeds such as phytic acid (38). Therefore, the solubility characteristics of proteins in their isolated form differ in a remarkable measure from that of the proteins contained in the seeds as shown by Fontaine *et al.* (39) for peanut and cottonseed proteins.

Some seed globulins can be fractionated. Two globulins, legumin and vicilin, which differ in solubility and in sulfur content have been isolated from peas (40). Two distinct globulin components have also been found in peanuts (41, 42), and in soybeans (43, 44). The globulins of leguminous seeds differ from the cereal prolamins in having a lower content of glutamic acid and a relatively high content of arginine, leucine, isoleucine, and valine. Legume seeds are low in content of the sulfur amino acids; their low value in promoting wool growth in sheep is attributed to sulfur deficiency (45).

Data on the influence of varieties, growth conditions, and ripening on protein content and amino acid composition of legume seeds are scarce. An important paper by Danielsson (46) may be mentioned in this respect. This author has studied pea seeds at different stages of growth. The globulin fraction increases during ripening, but the two globulins, legumin and vicilin, are synthesized at different rates; and the low-molecular nitrogen compounds decrease during ripening. The reverse process, breakdown of the globulins, takes place during their germination; hence, the globulins in leguminous seeds have the same functions as the prolamins and glutelins of the cereals; i.e., they are reserve proteins.

4. Proteins of Other Seeds

Only a few investigations on proteins of other seeds have been carried out, and these are reviewed in later chapters. Our knowledge of coconut protein is very limited; the globulin obtained from coconut (47) can be separated into two components by sedimentation analysis (48). The amino acid composition

of the meal is given in another chapter. Amino acid analyses of the globulins of hemp seed (edestin), of tobacco seed, and of the seeds of pumpkin, squash, watermelon, and cucumber have been reported by Smith and Green (49). According to these results the amino acid compositions of these seed globulins were found to be similar. The chief characteristic of their composition is the high arginine content which may be responsible for their low solubility in water and in salt solutions. Globulins have been prepared from citrus seeds (50), too, but information on their composition is lacking. Castor beans contain a toxic globulin, ricin (51). Its resistance to digestion by proteolytic enzymes is probably responsible for the property of being toxic on oral intake, unlike most other toxic proteins of plant or bacterial origin which are toxic only by injection. The mechanism of its toxic action is not yet known. (See also Chapter 31.)

III. LEAF PROTEINS

Leaf proteins comprise the enzymatic systems responsible for the photosynthesis and the wide range of other metabolic processes of which the higher plant is capable. The practical importance of the leaf proteins lies in the fact that these proteins offered in green forages to animals form a considerable part of their protein ration. Most forage crops belong to two plant groups—the grasses and the legumes; the latter, at comparable stages of growth, have a higher protein content.

1. Effect of Age and Conditions of Growth

Protein content of green plant species is more strongly influenced by growth conditions and state than that of grains. That the percentage of protein in green forage crops diminishes greatly during growth is well known (52) and of paramount importance for their evaluation for feeding purposes. Such a characteristic decrease during plant growth is exemplified by the data of Fagan (53) on Italian rye grass; the dry matter contained 18.5% protein at two weeks, 12.1% at six weeks, and 6.9% at ten weeks.

The leaf is richer in protein than the stem, and the proportion of leaf to stem changes with age. These two facts contribute to the unusual decrease in protein content during plant development as shown in the following tabulation.

PROTEIN CONTENT OF PERENNIAL RYE GRASS, LEAF, AND STEM EXPRESSED AS PERCENTAGE OF DRY MATTER (53)

Young		Mature	
Stem	Green leaf	Stem	Green leaf
%	%	%	%
15.5	24.8	10.4	17.7

Other factors, such as season of growth, time of day of cutting, and variations in soil moisture and temperature, influence the protein content of green plants. Table II, taken from the work of Shutt *et al.* (54), shows the changes in protein content of meadow foxtail under different systems of cutting. The plants cut at shorter intervals are richer in protein content.

An increase in the protein content of forage crops, especially at later stages of growth, may be observed to result from nitrogenous manuring. Lewis (55) was able to increase the protein content of hay crops

TABLE II
PROTEIN CONTENT OF GRASSLAND HERBAGE AS AFFECTED BY FREQUENCY OF CUTTING^a

Frequency	Season		
	1927	1928	1929
	Per cent of dry matter		
Cut weekly	21.2	28.9	29.2
Cut fortnightly	18.6	22.5	26.4
Cut every third week	17.2	20.7	22.9
Cut as hay	10.2	13.3	11.2

^a According to F. T. Shutt, S. N. Hamilton, and H. H. Selwyn, *J. Agr. Sci.* **18**, 411 (1928); **20**, 126 (1930); **22**, 647 (1932).

1.5 to 3.0 percentage units by fertilizing with nitrogen shortly before cutting.

2. Isolation of Protein

The preparation of native protein from leaves has proved to be much more difficult than similar preparations from seed material. The leaf is usually highly hydrated, its protein concentration being correspondingly low. Protein of fresh leaves makes up less than 5% of the total fresh weight as compared to seed protein which may make up 40% or more of the seed weight.

Different modes of extraction have been proposed for the preparation of leaf proteins (2, 5-7, 56). They can be removed from the plant materials by the following methods:

1. Finely ground fresh material is macerated in the presence of water under pressure (57, 58). The leaves can be cytolyzed with ether-water before being macerated. Such use of ether helps to liberate protein from the chloroplasts, thereby increasing their yield on extraction. Highly efficient and complete extraction of protein from leaf tissue can be obtained by use of the "colloid mill," a high-speed centrifugal grinding machine first recommended for this purpose by Wildman and Bonner (59).

2. Leaf material is extracted with mildly alkaline buffers in the presence of alcohol (60) or with very dilute alkali, according to Crook (61), who has worked out exact conditions for an almost quantitative extraction of protein from leaves, a method which has been used successfully by Kemble and Macpherson (62).

3. Leaf proteins are extracted by use of hot dilute mineral acids or 90% formic acid (63).

Leaf proteins are of minimum solubility in the pH range 4 to 5 and can be flocculated from aqueous solutions with the aid of ammonium sulfate or ethanol, or by adjustment of the hydrogen-ion concentration. The protein preparations are contaminated by varying amounts of polysaccharides or polyuronides, depending on the method. They are almost free of lipids because, in the course of the preparation, alcohol-ether or acetone is employed for their separation. Fresh leaves must be used, since protein in leaves dried even at fairly low temperature is almost insoluble in water or mildly alkaline buffers. Kolousek and Coulson (64) isolated proteins from hay by extraction with weak sodium hydroxide solution; but a part of the hay protein cannot be dissolved in weak caustic soda solution—it seems to be denatured by the drying process.

In some instances the leaf proteins can be separated into three groups, depending on their cellular origin: protoplasm, cytoplasm, and chloroplasts. The chloroplastic protein, which is associated with the green plant pigment, and the cytoplasmic protein, which would be free of pigment, are found in the greatest proportions. The vacuole fluid contains a little protein in solution. Laboratory-scale methods for the separation of these fractions have been reported (4, 7, 65). The three protein fractions of the same plant differ in their physical properties but are similar in their amino acid composition.

Methods for the large-scale production of edible protein from fresh leaves have been proposed by Pirie (66). The necessary machinery is complicated, but this author sees good prospects for future development owing to the high nutritive value of leaf proteins and the chance to use them for human nutrition.

3. Amino Acid Composition

The essential amino acids are present in the leaf proteins in amounts comparable, with some exception, to those of casein. Protein preparations obtained from different plant species do not show great differences in their amino acid composition. Relatively low cystine and methionine values were found in proteins prepared from leguminous forage crops (67) compared with those reported for the other plant families. The amino acid composition of the bulk protein of the leaves for any one species varies little with age of plants, with manurial treatment, and with climatic conditions; a variation hardly significant occurs between the proteins isolated from lucerne at different stages of growth (68). Lugg and Weller (69) found that the protein contained in senescent leaves of subterranean clover was of significantly lower

methionine and higher cystine content than that in the leaves of the mature plants.

The composition of the proteins contained in leafy vegetables is similar to that of green forage proteins, and the changes in protein content of vegetables and forages caused by different growth conditions are similar. Essential amino acid analyses of protein of vegetables have been published by Baptist (70). A protein of spinach, called spinacin, was isolated by Chibnall (71). Data on leaf proteins of plants not serving as human or animal food are scarce. The amino acid composition of leaf proteins of various flowering plants was compiled by Lugg (5). Leaf proteins are also discussed in Chapters 9 and 25.

IV. PROTEINS FROM OTHER VEGETATIVE PARTS OF FLOWERING PLANTS

Few investigations have been published on the proteins in roots, tubers, and fruits. Careful work however, has been done on the protein in potatoes, because potatoes are a valuable source of protein in human food. Feeding trials indicate that potato protein is of relatively high nutritive value (72). The two potato proteins, tuberin and tuberinin, have been investigated by Groot *et al.* (73). Only about one-half of the nitrogen of the potato is in the form of protein. Neuberger and Sanger (74) have shown that not only the total nitrogen of the potato, but also the relative proportion of protein and non-protein nitrogen, varies greatly from one variety to another. It was also demonstrated that the nitrogenous materials are not equally distributed throughout the tuber; thus the insoluble nitrogenous material is present mainly in the skin and cortex.

Sweet potatoes contain 2 to 3.5% protein (75), of which ipomoein, a globulin, constitutes the greater part. The proteins of turnips, present in only a small percentage, are similar in their amino acid composition to leaf proteins (76). A paper on the distribution of the different nitrogen fractions in turnips, kale, kohlrabi, and parsnips was published by Davies as early as 1927 (77). Carpenter and Smith (78) described the isolation of a crystalline globulin from tomato juice.

Fruits also contain limited amounts of proteins together with other non-protein, nitrogenous substances (79). Nelson *et al.* (80) found that half of the nitrogen in orange juice was present in the form of free amino acids and the other half as protein. Changes in the protein content of fruits during ripening have been studied, a decrease being found in peaches (81), plums (82), and figs (83), and an increase in grapes (84). A study on different protein fractions of the avacado has been published by Jones and Gersdorff (85).

The nuts are comparatively rich sources of protein. Almost no further research was carried out on the proteins of almonds, hazel nuts, and walnuts after the classical work of Osborne, except for analyses of the total protein content published in tables of food composition (16). Proteins of the tropical cashew nut have been isolated by Damodaran and Sivaswamy (86).

Bark contains cytoplasmic proteins; Briggs and Simonovich (87), who have isolated these proteins, have shown by electrophoretic examination that they consist of five distinguishable components. Development of cold resistance of trees is associated with an increase in three of these compounds.

Fresh rubber latex contains about 1% protein; the presence of protein in latex is an important factor controlling its colloidal stability. Properties and composition of this protein have been described by Archer and Sekhar (88), who succeeded also in separating electrophoretically distinct protein fractions.

Pollen proteins (89) are rich in essential amino acids. This advantageous chemical composition enables the pollen to serve as protein food for bees and other insects.

V. PROTEINS OF LOWER PLANTS

These proteins are discussed in detail in Chapters 29 and 30 and hence they will not be dealt with here. Suffice to state that as proteins of metabolic tissue they have a high intrinsic nutritive quality similar to that of leaf protein. In many instances, however, they are accompanied by indigestible material, bitter principles, etc., which interfere with their full utilization.

VI. THE NON-PROTEIN NITROGEN OF PLANTS

In a summary of his work published in 1906 Schultze (90) reported the isolation of ten amino acids, amides like glutamine and asparagine, and other nitrogenous compounds from seeds and seedlings. Considerable progress has been achieved since then in the study of non-protein nitrogen, particularly recently by means of modern physical and chemical methods (91, 92). Fifteen to twenty-five per cent of the total nitrogen in fresh leaves consists of free amino acids, amides, peptides, nitrates, ammonium salts, etc., and a number of minor constituents of relatively little importance as nutritional sources of nitrogen, such as nitrogenous bases (purines, choline, betaine, and also alkaloids), chlorophyll, and glycosides. Urea can be synthesized in mushrooms (*Agaricus campestris*) from ammonium salts according to Ivanov (93); this interesting result has not been investigated further. The free amino acids and peptides contained in different pasture plants have been investigated by Bathurst (94). It is a notable fact that free amino nitrogen contained in pasture plants originates from relatively few amino acids, i.e., the dicarboxylic acids and their amides (glutamine and asparagine), alanine and serine. The predominance of non-essential amino acids readily explains the poor nutritional results obtained when plant non-protein nitrogen is substituted for protein in non-ruminant nutrition.

According to Kolousek and Coulson (68) the non-protein nitrogen fraction varies more in composition with time of cutting and use of fertilizers than does the protein fraction. The more favorable are the growth conditions, the higher is the content of the non-protein nitrogen fraction as well as the total nitrogen content. Water-soluble nitrogenous

substances tend to accumulate in phosphorus-deficient plants. This effect may be attributed to a decreased rate of synthesis of proteins from amino acids. The ratio of non-protein nitrogen compounds to proteins falls off markedly with increasing age and fiber content of the plant. Kolousek and Coulson studied the fluctuations in the free amino acid content of alfalfa cut at different stages of growth and found that the free amino acid and amide content is at a minimum at late budding. It is suggested that the increase of this fraction after budding is due to protein degradation preceding translocation of simpler nitrogenous materials which are required for the process of flowering.

Small amounts of rare free amino acids were also detected in various plants (95, 96). The presence of compounds such as γ -aminobutyric acid and β -alanine in leguminous forage may be mentioned; these compounds can be obtained from glutamic and aspartic acids by decarboxylation. The discovery of heterocyclic compounds such as pipecolic acid in leguminous plants is worthy of note (95, 96). This substance can be derived from lysine by cyclization with the elimination of ammonia.

Non-protein nitrogen is also contained in considerable quantities in the lignins of the cell walls of green plants (97), whereas wood lignins are practically nitrogen-free. Lignins prepared from grasses contain 1.2 to 1.6% nitrogen, and those from leguminous forage have 2.9 to 3.4% nitrogen. The presence of nitrogen in plant lignins is not due to accidental contamination with proteins, but nitrogen is a characteristic component of the lignin molecule itself. The lignin nitrogen is present as tertiary nitrogen, probably in a cyclic compound.

The proportion of soluble nitrogen compounds in the total nitrogen of forage plants rises markedly on ensiling (98). This breakdown of proteins is not disadvantageous from the point of view of ruminant nutrition. The chemical forms in which nitrogen occurs in green plants are not greatly changed by hay making (68). Enzymatic destruction of protein to a small extent may take place during wilting (62), and if hay is exposed to rain, some of the non-protein nitrogen will leak out.

Free amino acids and amides often form a greater percentage of the total nitrogen contained in fruits (99) and tubers than in green plants. Hydrolysis of proteins occurs in fruits during storage and leads to the accumulation of free amino acids; low temperature and immaturity of fruit inhibit these changes (100).

The amount of non-protein nitrogen in mature seeds is generally small, and its nature has seldom been investigated. The presence of greater amounts of non-protein nitrogen in mature seeds of galega and fenugreek seems to form an exception (101). A considerable part of the nitrogen contained in green leguminous seeds, on the other hand, is non-protein nitrogen as was shown for green peas (102).

VII. SYNTHESIS OF AMINO ACIDS AND PROTEINS

Plants are distinguished by their being able to synthesize proteins from simple inorganic nitrogen compounds. Nitrate formed by bac-

terial oxidation of decaying plant and animal material in the soil or added as a fertilizer is the principal form of nitrogen used by most species of higher plants and in general undergoes reduction after absorption. This process is accompanied by a depletion of stored carbohydrate; i.e., the respiration rate of the plant increases, and the energy derived from such respiration is used, in part, for the reduction of nitrate.

Keto acids, intermediates in the metabolism of carbohydrates, are the principal acceptors of nitrogen, thereby linking carbohydrate metabolism with protein synthesis. For example, hydroxylamine can react with α -ketoglutaric acid to yield α -oximinoglutaric acid which can then be reduced to amino acid. Virtanen (96) detected various keto acids in plant tissues; their reaction with hydroxylamine makes possible the formation of different amino acids, and hence the great variety of proteins in plants. It can be assumed that part of the nitrate is also reduced directly to ammonia which can react with organic compounds in the plants.

The leaf is an active site of protein synthesis. Continuous degradation of proteins balanced by continuous resynthesis takes place. It seems that tissue proteins, once formed, are not static substances and that a cyclic process of protein synthesis and degradation is in operation throughout the life of the plant. The balance between simple nitrogen compounds and protein nitrogen is shifted far in the direction of non-protein nitrogen in the dividing cell; growth by cell enlargement is accompanied by protein synthesis. Moreover, as was pointed out previously, the nature of proteins contained in the leaves changes with age; the carbon-to-nitrogen ratio is lower in young leaves than in the older ones—the nature of the protein synthesized changes from proteins rich in basic nitrogen constituents, such as the nucleoproteins, to proteins that are relatively poor in these fractions. Capacity for protein synthesis appears to decrease with age of the leaves; and this effect may be connected with changes in the enzyme systems.

Active protein metabolism takes place in germinating seeds. Germination involves the mobilization of the protein stored in the seed which is hydrolyzed to amino acids and amides, which are then transported to the growing tissues of the seedling where they are synthesized into protein. Since the protein of the seedling differs in amino acid composition from that of the seed protein, it is clear that degradation through proteolysis and resynthesis must occur during germination. Synthesis of considerable amounts of asparagine and glutamine takes place during germination. This amide formation can be regarded as a mechanism for detoxification of the ammonia liberated by proteolysis

and constitutes an effective way for the translocation of the seed nitrogen. This same detoxifying process takes place in the beet with the formation of glutamine; the glutamine content of the beet increases rapidly when great amounts of ammonium sulfate are applied to the soil (103).

The entire subject of nitrogen metabolism in green plants is a field of active research and has been reviewed on several occasions in recent years (104).

VIII. ENZYMES

Aside from the storage proteins, an important group of plant proteins are the enzymes. They are reviewed briefly here; there are many extensive reviews which deal with this subject in greater detail (105). All metabolic processes occurring in plants, and which involve synthesis or degradation, are connected with enzymes. Many plant enzymes have been purified and obtained in crystalline form, among them urease, papain, ficin, ascorbic acid oxidase, peroxidase, and α - and β -amylase.

Although there are individual differences, the general character of metabolism in plant and animal tissues is similar, and many enzymes performing similar functions may be isolated alternatively from plant or animal sources. Nevertheless certain enzymes from plants have attained recognition, for historical reasons or otherwise, as uniquely plant enzymes. Among these are proteolytic enzymes, the best known of which is papain. Papain is obtained from the small softwood tree, *Carica papaya*, cultivated in tropical countries. This enzyme makes up as much as 50% of the dry weight of the latex. Papain can be used technically for tenderizing meat and for raising the nutritional value of proteins by enzymatic predigestion (106). The structure of crystallized papain has been clarified by Smith and others (107–110). Ficin is a proteolytic enzyme present in the latex of fig trees, and bromelain occurs in the pineapple plant (111). Proteolytic enzymes contained in rarer plants are listed in a review by Langlyke *et al.* (112).

It has been reported that proteolytic enzymes are present in the seeds of grains (113); the enzymatic activity of seeds seems to be a measure of their vitality, and the amount of enzymes increases during sprouting of seeds (114). Protease activity has also been found in green leaves (115).

A class of enzyme inhibitors of interest in connection with utilization of proteins of leguminous seeds is the trypsin inhibitors. These have been reviewed (116) and are also discussed in Chapters 5 and 14.

Urease is an enzyme which is important in use of certain foodstuffs,

particularly for ruminants. It catalyzes the conversion of urea into ammonia and carbon dioxide and has been found in jack beans, soybeans, and melon seeds, and in many species of bacteria, yeasts, and molds (117) as well as in rumen contents (91). It is of interest that urease was the first crystalline enzyme to be prepared. (See also Chapters 5 and 14.)

IX. DIFFERENCES BETWEEN PLANT AND ANIMAL PROTEINS

It has been assumed that animal proteins are superior to plant proteins as food for non-ruminants and man. Although this may be true in many instances, the general statement is open to debate as has been pointed out in Chapter 1. There are many plant proteins which are deficient in certain essential amino acids, notably lysine and methionine; they are inferior to animal proteins. But there are also plant proteins which are adequate in composition of essential amino acids. On account of amino acid composition alone, such proteins should not be inferior to animal proteins.

There is the thought that factors other than amino acid composition might be responsible for differences between animal and plant proteins. Bondi and Birk (118) studied the rates of liberation of amino acids by *in vitro* digestion with proteolytic enzymes and were unable to find any major differences between the two classes of proteins. They did find, however, that proteins of plant origin contain a fraction which dissolves in buffer solutions at pH 8, is precipitated by trichloroacetic acid, and is only slightly attacked by pancreatic enzymes. Animal proteins lack this fraction. These authors assume that the existence of this protein fraction, which is more stable to enzyme attack, may affect the nutritional value of the plant proteins.

Another difference between plant and animal protein feeds has also been attributed to the presence of peptides such as streptogenin (119); these peptides which are characteristic of animal protein feeds are stable to digestive enzymes and are absorbed from the intestinal tract without any preliminary breakdown. It may be pointed out, that nutrition experiments carried out recently do not give supporting evidence for the suggestion that animals require streptogenin (120, 121).

One major difference between animal and most plant proteins is not in the proteins themselves but in the accompanying materials, among which is the so-called "animal protein factor" (A.P.F.) (122). Vitamin B₁₂, cobalamin, is probably the major component of A.P.F., but this factor contains at least two other substances. The cobalamin content of the different protein feeds is given in Table III where the difference in content between plant and animal feeds is clearly apparent. It is

TABLE III
COBALAMIN CONTENTS OF DIFFERENT FEEDSTUFFS^a

Feedstuff	Cobalamin content (γ /g.)
Red fish meal	62
Sardine meal	154
Herring meal	260
White fish meal	95
Meat meal	36
Liver meal	310
Crude casein	104
Wheat	1
Corn	0
Oats	3
Soybean meal	2
Alfalfa meal	3
Dried yeast	1

^a From W. Bolton, in "Progress in the Physiology of Farm Animals" (J. Hammond, ed.), Vol. 1, p. 139. Butterworth's, London, 1954.

known, however, that bacteria synthesize this material so that this characteristic difference is eliminated by supplementing plant proteins with cobalamin from microbiological sources. (See also Chapter 1.)

Other differences, if any, between plant and animal proteins, such as characteristic sequence of amino acids, must await progress of research on the structure of proteins derived from plants.

X. SUMMARY AND CONCLUSIONS

This chapter deals with general properties of plant proteins, principles of their classification, and modes of their isolation. A short description is given of different groups of plant proteins, including the proteins of leguminous seeds, cereal grains, some rarer seed proteins, and the leaf proteins. Attention is given to simpler non-protein nitrogenous compounds occurring in plants which are recognized as intermediate products in the biosynthesis of plant proteins.

The plant proteins are of manifold amino acid composition and have a well-organized structure in order to fulfill their important functions in plant metabolism. It is to be expected that in the future new and improved methods for the processing of plant proteins for food will be developed, based on most recent progress in chemistry and biochemistry of plant proteins. The new products which will arise will be utilized more effectively by man and animals than our customary plant protein foods and feeds. A special task will be to modify plant

proteins, particularly by isolation and supplementation, in order to provide additional sources of good-quality protein less expensive than the animal protein foodstuffs. (See also Chapters 11 and 13.) Uses of plant proteins for production of industrial products are discussed by Smith in Chapter 10.

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CHAPTER 4

PROCESSING OF OILSEEDS

H. D. FINCHER

I. HISTORICAL

It appears that the first crude attempts at processing oilseeds occurred before the dawn of recorded history. Early Hindu writings refer to pounding cottonseed and boiling the pounded material to recover oil (1). The ancient Chinese are said to have obtained oil by reducing oilseeds to meal under an edgestone, heating the meal in an open pan, and pressing it in a wedge press (2). At later dates several types of lever presses and hand-powered screw presses were invented by the Greeks and Romans (3). These early efforts were prompted by the desire for oil, but it seems likely that interest in and utilization of the oilseed cakes which are the residues after oil extraction followed soon thereafter.

Development of the hydraulic press marked a long stride forward in the commercial processing of oilseeds; such a press was patented in England by Joseph Bromah in 1795 (4). Hydraulic processing became the predominant method in the oilseed industry and continued so until well into the twentieth century. Its higher labor requirement and less efficient recovery of oil have caused it to lose ground to continuous methods.

Continuous methods of processing, which include screw-pressing and solvent extraction, were developed early in the twentieth century (3); however, only a few continuous systems were operating prior to World War I. Screw-pressing became widely used in the United States after World War II; solvent extraction followed quickly, and it could almost be said that the two processes grew up together. Owing to the combined efforts of equipment manufacturers and processors, the efficiency of continuous equipment has improved rapidly. The major problems have been resolved, and today continuous methods are accorded general acceptance.

It is interesting to note that most of the European screw presses

were developed to forepress seeds of high oil content and prepare them for finishing by hydraulic pressing or solvent extraction. Those developed in the United States were primarily for complete pressing (3).

Since oilseed meals form such a large proportion of processed vegetable proteins, oilseed processing is a subject of pertinence in many of the chapters that follow. This general discussion is intended as an introduction to the subject. Applications to specific oilseeds are described in many of the chapters in Part II.

II. HYDRAULIC PROCESSING

The preparation of oilseeds, or the oil-bearing portion thereof (meats), for hydraulic pressing usually takes place in three steps: first the seeds are crushed between heavy rolls, the material is then cooked, after which it is dried to the optimum moisture content for pressing.

The primary objective in the crushing operation is a reduction in particle size or particle thickness, and it is customary to produce flakes of 0.008 to 0.012 inch in thickness. Having the material in such a finely divided state contributes to uniform cooking and drying. Crushing rolls are usually stacked, and the seeds to be crushed make several passes between pairs of rolls.

There are several reasons for the cooking step (5-8). Investigators are in general agreement on the following objectives:

1. To rupture or finish rupturing of oil cells.
2. To increase the fluidity of the oil by an increase in temperature.
3. To coagulate or granulate the protein aleurone grains. This facilitates a separation of the oil from the proteinaceous and other materials.
4. To "precipitate" phosphatidic material in order to produce oil of lower refining loss.
5. To destroy molds and bacteria.
6. Additionally for cottonseed, to detoxify free gossypol by converting it to the "bound" form. (See also Chapter 17.)
7. To inactivate enzyme systems which have adverse effects on the quality of oil and meal.

Cooking may be carried out in a single vessel or in a series of vessels. Frequently, several vessels will be stacked one on top of the other with the meats flowing through them in series; such an arrangement is called a stack cooker. All cookers are equipped with mechanical agitators for stirring the material during the cooking operation. The

cookers are steam jacketed for heating, and additional heat may be supplied by injecting live steam into the mass.

The three important variables in cooking are temperature, duration, and moisture content and their adjustment plays an important role in determining the quality of the protein in the finished meal. Low temperature, low moisture, and a short cooking time favor the production of high-quality protein. The opposite conditions tend to denature some of the protein, reduce its solubility, and lower the nutritive value. Protein quality is not the sole concern of the processor, however, and processing conditions will be dictated by oil quality and extraction efficiency as well as by protein quality.

Considerable variation will be found in the conditions employed on any one oilseed, and these variations become even greater when several types of oilseeds are considered. In hydraulic processing it has become customary to consider cooking and drying as a single operation, which means that the latter part of the cooking cycle is really a drying step. If we wish to consider these steps separately, it can be said that hydraulic cooking temperatures for the various oilseeds will range from 150° to 220°F. and drying will be carried out at temperatures 10° to 20°F. higher than cooking temperatures. Moisture content during cooking will vary from 6 to 15%, and the length of time will range from a short cook of 15 minutes to as long as 120 minutes. The material is dried to the optimum moisture for efficient extraction; this moisture content will vary for different oilseeds. In general, the moisture for pressing will range from 4 to 9%, materials of high fiber and low protein content being pressed at the higher moisture levels.

As the material leaves the cooker, it must be formed into cakes for handling in most types of presses. A mechanical device known as the cake former spreads a layer of the material over a press cloth which has been placed in the bed of the former. The ends of the cloth are folded over to cover the cake completely, and slight pressure is applied to compact and form the cake so that it can be moved to the press by hand. A flat metal "pan" is slipped beneath the cloth-wrapped cake, and it is transported to the press.

The hydraulic box press consists of twelve to sixteen "boxes," each of which receives one cake. The boxes are linked together one below the other, and beneath the boxes is the hydraulic ram for exerting pressure. After the boxes are filled, valves are opened to admit hydraulic fluid to the cylinder, and the ram rises to compress the cakes for oil expression. The maximum pressure on the cakes is usually about 2000 p.s.i.; however, some presses have been constructed with larger rams for reaching nearly twice this pressure. After a period of

20 to 60 minutes, the pressure is released, the cakes are removed by hand, and the cloths are stripped off for reuse.

Oil content of the pressed cake will depend on the type of seed, the length of time under pressure, and the preparation conditions; it may vary from below 5% to above 8%. The quality of the protein in the cake will be determined largely by the cooking conditions employed.

III. SCREW-PRESSING

Preparation of oilseeds for screw-pressing is similar to that for hydraulic press operation and normally includes the steps of crushing, cooking, and drying. There are some differences worthy of note, however, and on certain types of seeds one or more of the above steps may be omitted.

In the majority of installations the first preparation step will employ crushing rolls. In some instances, however, a grinding operation employing attrition or hammer mills will be substituted. This step may even be eliminated and the whole seed passed directly to the cooker as in the processing of sesame seed.

Processing temperatures are higher than for hydraulic operation and may reach 260°F . in cooking and 280°F . in drying. These high temperatures can be detrimental to protein quality, particularly if maintained for extended periods in the presence of high moisture.

Efficient operation of the screw press or Expeller* requires that the material be dried to a lower moisture content than required for the other extraction methods. Moisture content of the meats entering the press will normally be 3% or less and will be determined by extraction efficiency and operating characteristics of the machine. Drying may be carried out in one or more stages of the cooker, or may be done in a separate vessel referred to as a conditioner.

In the actual pressing operation the oil-bearing material is forced through the barrel of the screw press by heavy screws. Figure 1 shows the cage or barrel of a screw press that has been thrown open to reveal the heavy shaft (A) and flights making up the screw. Note that the flights are not continuous but are broken at intervals. This permits the insertion of bars or "keeper knives" which prevent the material from turning with the screw and ensure its being propelled forward through the barrel. Friction within the barrel causes pressures of several thousand pounds per square inch to be developed.

* Expeller is a term used by the V. D. Anderson Co. to describe screw presses of their manufacture. In a considerable portion of the literature on oilseed processing and even in some trading rules this term has been used synonymously with the term screw press.

The barrel is built up of a series of horizontal bars which are separated by spacers to allow the oil to escape as it is squeezed from the mass flowing through the barrel. The openings between the bars are in the range of 0.005 to 0.030 inch. Two horizontal lines of barrel bars are easily visible in Fig. 2 between the points (*D*) and (*E*). Additional bars making up the barrel can be seen by closer inspection.

The material being pressed is in the barrel for less than 2 minutes, but during this time it is subjected to relatively high temperatures due

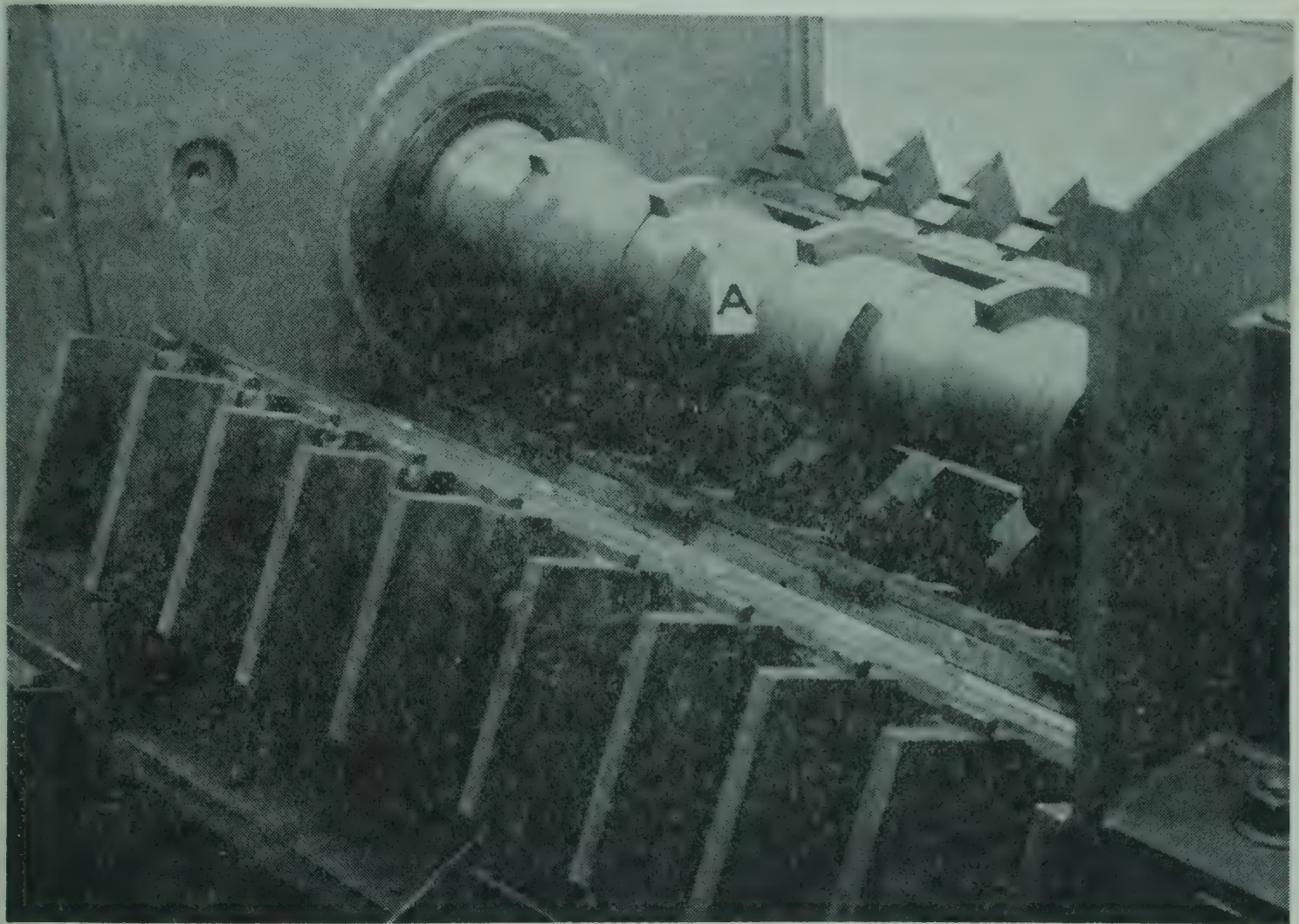


FIG. 1. Screw press cage and shaft. (Courtesy of Rose, Downs & Thompson, Ltd.)

to heat generated by friction. To prevent extreme temperatures which would cause excessive damage to protein and oil quality, it is customary to cool the barrel by circulating some of the cooled product oil over it or by circulating water through passages in the supporting structure of the barrel. Flexible hose connections for cooling water are shown in Fig. 2 at several points, such as (*F*). In some machines the shaft carrying the heavy screws is hollow and additional cooling is obtained by circulating water through it. From a standpoint of protein quality alone, it would be advantageous to cool more than is normally done; however, as in the cooking operation, the processor seeks a balance between protein quality, extraction efficiency, and oil quality.

Screw-pressing is the most drastic of the extraction methods as far

as heat treatment of protein is concerned. By determining the power used to drive the screws and the time the meal remains in the press, it is possible to calculate the work done on the meal. There are indications that a correlation exists between the amount of work done on the meal and heat damage to the protein, the damage increasing as the amount of work increases. Extraction efficiency falls between that of hydraulic and solvent methods and results in a meal containing 2.5 to 4.0% of oil.

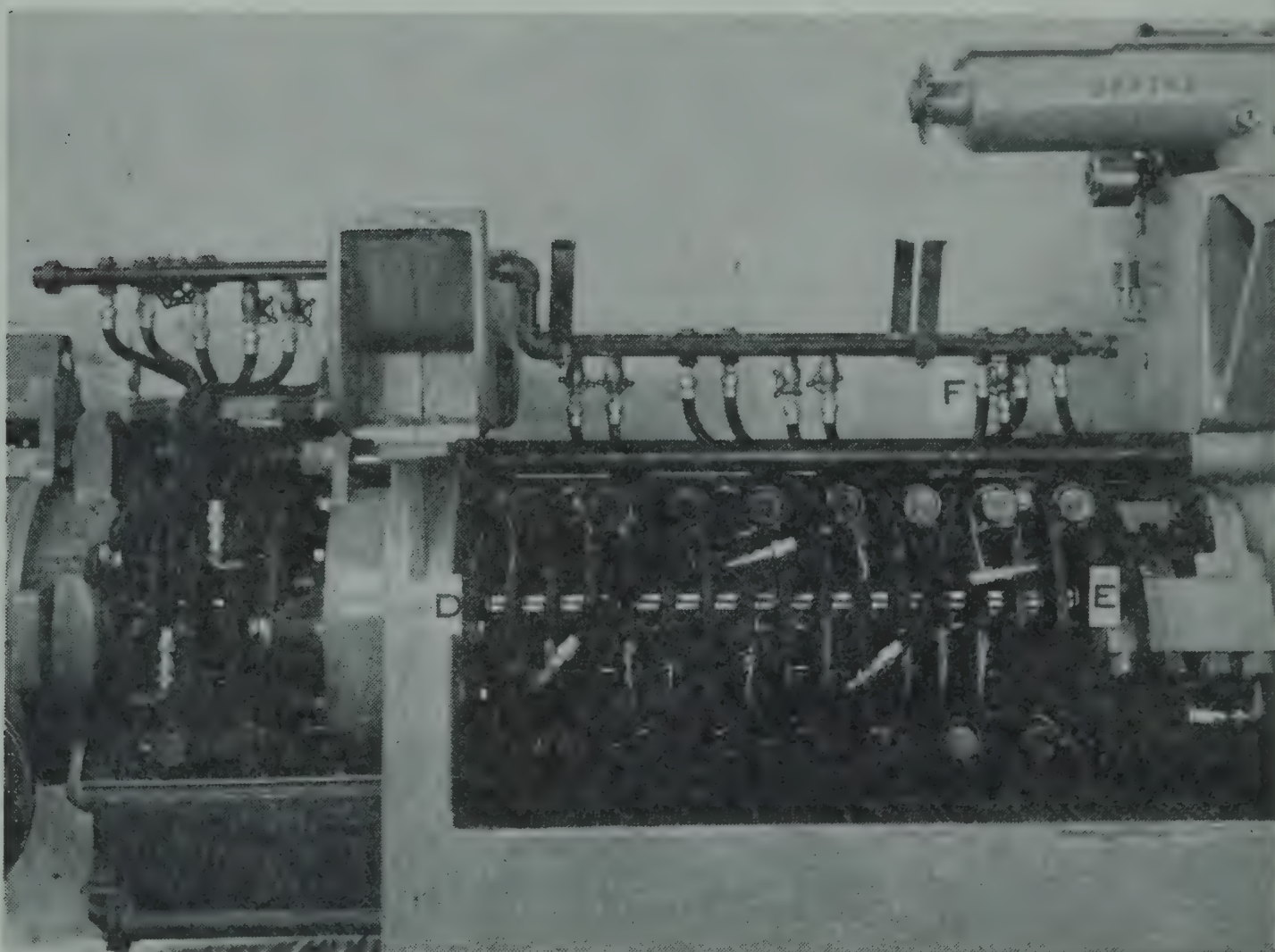
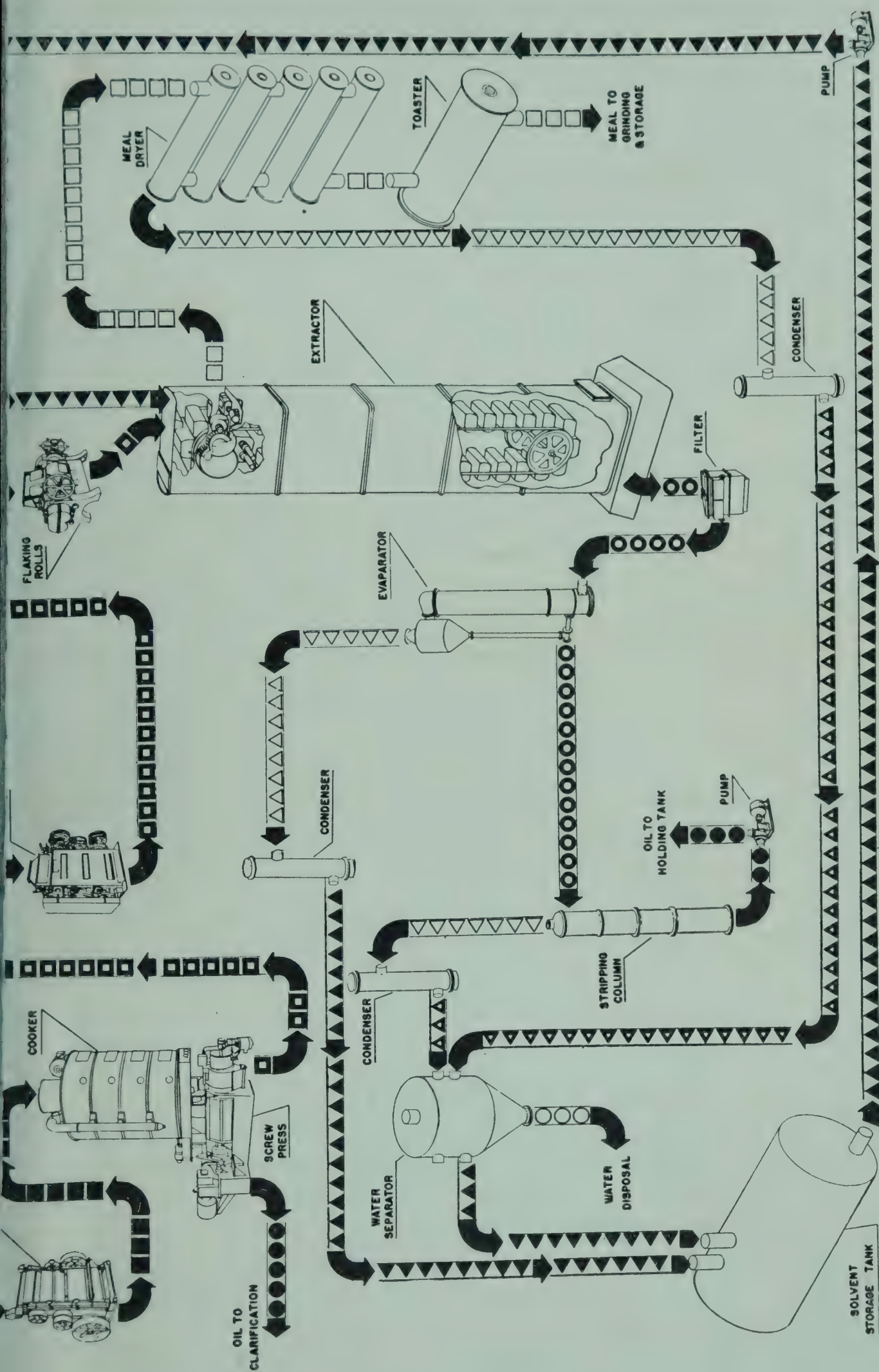


FIG. 2. Outside of screw-press barrel. (Courtesy of The French Oil Mill Machinery Co.)

IV. PREPRESS SOLVENT EXTRACTION

The choice between prepress solvent extraction and direct solvent extraction will usually depend on the oil content of the seeds to be processed. With seeds of high oil content it is more economical to remove some of the oil in a prepress operation. Another consideration is the fact that prepress plants are more versatile in their ability to handle various types of oilseeds. Some operators will choose a prepress plant even though a part of their operation may be on oilseeds of relatively low oil content such as soybeans. This is particularly true of operators of batch-type plants.

A general flow chart for prepress solvent extraction is shown in Fig. 3. It should be pointed out that variations from this general flow are not unusual. These variations will depend on the choice of the processor.



the type of seed being processed, and the type of solvent plant employed. Bagot (9-11) has reviewed the various methods and equipment for solvent extraction of cottonseed.

Preparation for prepress solvent extraction resembles closely that for hydraulic operation. Cooking and drying conditions are usually a little less severe, and this results in less heat damage to protein.

The presses used for prepressing are of the same type as those used for screw-press operation, although some machines are built primarily for prepressing. It is possible to convert a standard screw press to a prepressing machine by making slight modifications to the screws and barrel spacings and by increasing the speed of the screws. A prepress machine handles two to four times the tonnage handled by a standard screw press.

The prepressing step is almost identical to standard screw-pressing except that it is a relatively mild operation, pressures and temperatures developed in the barrel being somewhat lower. The material is usually pressed at a higher moisture content, and no attempt is made to reduce the oil content in the cake to the low figures obtained in normal screw-pressing. From 50 to 85% of the oil in the seed will be removed in prepressing, the amount depending on the original oil content of the seed and on the choice of the operator. Such treatment does not subject the protein to the extreme conditions encountered in screw-pressing. Some prepress machines are cooled by oil or water circulation, and others are not; the type of machine determines whether or not cooling is necessary.

Cake from prepress machines may go directly to the solvent plant without further treatment, it may be granulated for extraction, or it may be granulated and formed into flakes by passing through flaking rolls. The method of handling will depend on the type of extractor used and on the preference of the processor and is not important in determining protein quality.

In the solvent plant the prepressed cake is contacted by a solvent which extracts most of the remaining oil. In a continuous-type plant the material flow is continuous and the flow of solvent is usually countercurrent to the flow of cake.

Continuous solvent-extraction plants can be divided roughly into two groups: (1) the total submergence types, and (2) the percolation types. In the submergence types, as the name implies, the material to be extracted is submerged in a bath of solvent. In the percolation types, the solvent percolates through beds of the material to be extracted. One such extractor is shown in Fig. 4. In this particular extractor the material to be extracted is carried by large baskets having perforated bot-

toms. As a basket is filled at the top of the extractor a weak solution of oil in solvent (called miscella) which has been collected on the opposite side at the bottom of the extractor is sprayed over the solids. More oil is extracted as the liquid percolates through the bed of solids. It leaves

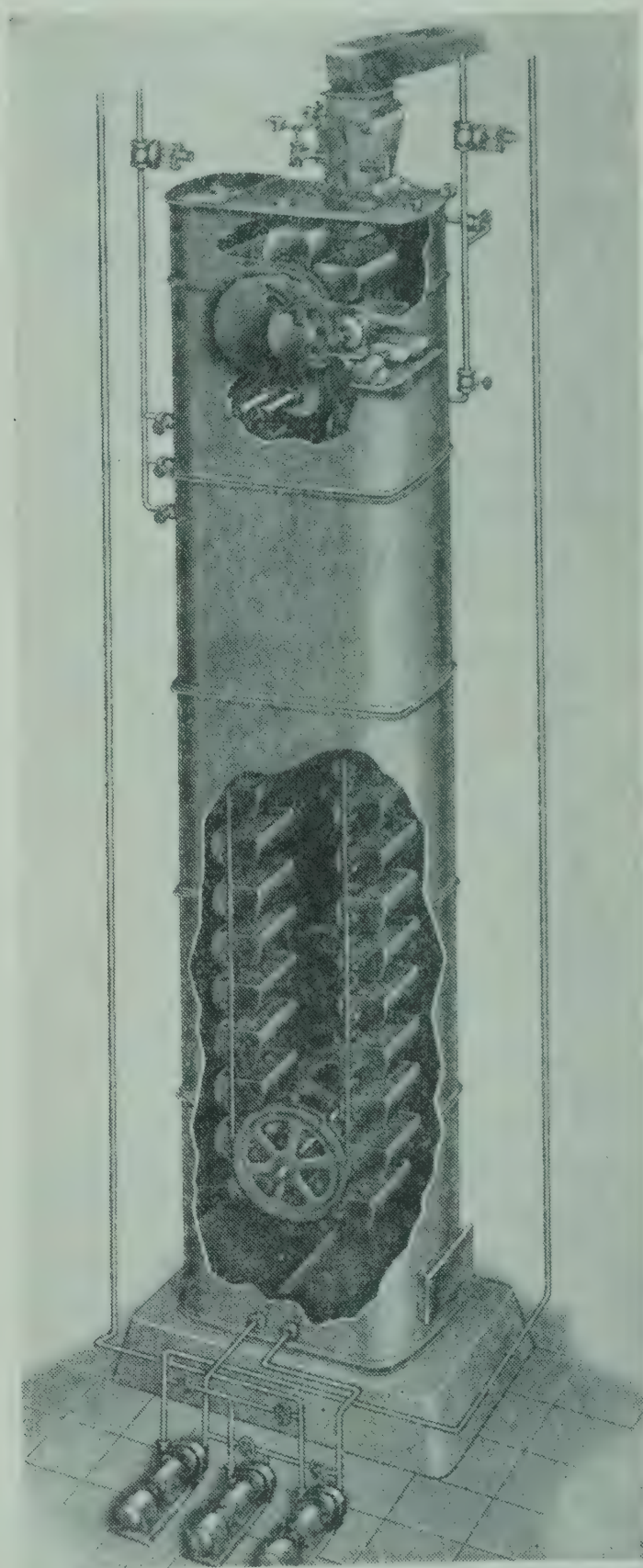


FIG. 4. Bollman-type extractor. (Courtesy of The French Oil Mill Machinery Co.)

through the perforations in the bottom of the basket, falling into the basket next below, and is finally collected at the bottom of the extractor. The baskets are traveling down on the side where they are loaded, and the flow of solvent is in the same direction as the flow of solids. On the opposite side the baskets are traveling up, and fresh solvent sprayed in

at the top percolates down counter to the flow of solids. (See also Chapter 14.)

Solids leaving the extractor carry entrained solvent amounting to 50 to 100% of the weight of solids. The meal is freed of this solvent in vessels known as dryers or desolventizers. These vessels are usually steam jacketed, the steam supplying heat for driving off the solvent, the solids being mechanically agitated to speed up the process. At least one manufacturer supplies desolventizers which utilize the heat of superheated solvent vapors for evaporating the solvent from the meal. Here the material to be desolventized is showered into an atmosphere of superheated vapors. Frequently, a small amount of live steam will be introduced near the point where solids discharge from the drying equipment. This sparge steam aids in sweeping away the last traces of solvent vapors. In the desolventizing operation, where hexane is used as the solvent, the meals are subjected to temperature of 180° to 220°F., which again is relatively low as compared with some of the temperatures in screw-pressing. Temperatures will be slightly higher when solvents of higher boiling point are employed. (See also Chapters 10 and 14.)

Some processors use batch-type plants where the material to be extracted is introduced into large steam-jacketed extractors holding several tons of cake. The material is given several consecutive washes with solvent to extract the oil, and the liquid is then drained off. Steam is admitted to the jacket of the vessel to supply heat for evaporating the solvent absorbed by the solids. At the end of the desolventizing period a small amount of live steam is sparged into the vessel to sweep out the last solvent vapors.

Residual oil in meal produced by the prepress solvent-extraction method will be 1.0% or less.

V. DIRECT SOLVENT EXTRACTION

In direct solvent extraction there is no prepress operation and all the oil is removed by extraction with solvent. This process is usually employed on materials having an oil content of less than 35%.

In Table I are listed the author's opinion of preferred methods for processing some of the more common oilseeds. In making these selections consideration has been given to economics and to problems of materials handling. Oilseeds which produce toxic meals, such as castor beans and tung nuts, have been omitted from this table.

The seeds or meats to be extracted may be cooked as for hydraulic or prepress operation; however, the usual procedure is to condition the material by mild heating and adjustment of moisture and then flake

by passing it through flaking rolls. Conditioning contributes to formation of a thin flake (0.010 inch or less) which is easily extracted and a tough flake which does not disintegrate while in contact with solvent in the extractor. On some materials of a fibrous or granular nature the conditioning and flaking steps may be omitted.

Equipment and procedures for extraction and desolventizing are practically identical to those for continuous solvent extraction after a

TABLE I
RECOMMENDED TYPE OF CONTINUOUS PROCESSING FOR THE MAJOR OILSEEDS

	Average oil content (%)	Preferred process ^a		
		Screw press	Prepress solvent extraction	Direct solvent extraction
Babassu	65 ^b	2	1	—
Brazil nuts	68 ^b	2	1	—
Copra	67	2	1	—
Coquito kernels	50	2	1	—
Corn germs	50	3	1	2
Cottonseed	33 ^b	2	1	1
Flaxseed	37	2	1	—
Palm kernels	48	2	1	—
Peanuts	48 ^b	2	1	—
Rice bran	15	3	2	1
Sesame	52	2	1	—
Soybeans	18	2	3	1
Sunflower	42 ^b	2	1	—

Numeral 1 indicates the first choice of method, numeral 2 the second choice, and numeral 3 the third choice.

^aOn the basis of dehulled meats or kernels.

prepress operation. It is customary to toast soybean meal after desolventizing in order to inactivate enzyme inhibitors and other heat-labile materials and improve the palatability of the meal. In general, the materials handled by direct extraction are too finely divided and too fragile to give satisfactory performance in the large batch-type extractors.

Except where cooking is employed prior to extraction, the direct solvent-extraction process presents less opportunity for protein damage than any of the other methods. Differences will not be great, but this process can be expected to produce protein quality slightly superior to that for prepress solvent extraction.

Residual oil in meal will average slightly higher than for prepress solvent extraction but will be near 1.0%.

VI. PROCESSING TRENDS

Recent years have seen a steady trend toward continuous operation where presses are employed. Some of the reasons for this trend are: (1) Extraction is more efficient. (2) Less labor is required. (3) No press cloth is required.

Continuous pressing does have the disadvantages of higher power usage and of requiring that the product oil be filtered.

The general trend in solvent extraction also has been in the direction of continuous operation. This is particularly true in the United States where the tendency has been to make all processes continuous as far as possible. Extraction efficiency is at least equal to or slightly better than for the batch process; labor requirements are lower for continuous plants.

In areas where relatively low labor rates exist some processors still prefer batch operation. Some of the reasons for this preference are: (1) Initial costs are lower. (2) Equipment is simplified. (3) Operation is less complicated and the effects of interrupted operation are less serious. (4) There are fewer moving parts where solvent leaks can develop.

Where prepressing is employed, this will usually be on a continuous basis even if the extraction step is a batch operation. Most often it is found advantageous to evaporate miscella continuously.

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CHAPTER 5

EFFECT OF HEAT ON PLANT PROTEINS

IRVIN E. LIENER

I. INTRODUCTION

Because of the quantity and quality of the protein which they contain, oilseed meals provide an economical and nutritionally valuable source of protein in the formulation of mixed animal feeds. Legumes and cereals likewise constitute an important dietary source of protein for many segments of the world's population, particularly where animal protein is in short supply or is forbidden by cultural or religious habit. In the United States, where no shortage of animal protein exists, almost 30% of the total dietary protein is derived from cereals and legumes (1).

In most instances, vegetable proteins are not consumed in their native state but in a form which has been subjected to some degree of heat treatment. Oilseed meals are by their very nature the products of processes designed to effect the removal of oil from the original seed, and the application of heat is an inherent feature of such techniques. Legumes and cereals intended for human consumption are cooked or processed to enhance their palatability and acceptance. As a consequence of such heat treatment, the nutritive value of the protein may be affected in a manner which varies not only from one protein to another but also for the same protein, depending on such factors as the temperature, the duration of heating, and the presence or absence of moisture.

Recent years have witnessed a tremendous interest in the potential use of vegetable proteins for a wide variety of industrial purposes (2, 3). Important considerations here are the chemical and physical properties of the protein as affected by processing, since these properties markedly influence the utility of the protein. Moreover, a knowledge of the physical-chemical changes induced in proteins by heat may lead to a better understanding of the effect of heat on the nutritive properties of the protein. For these reasons, this chapter will not consist of a

mere documentation of recorded facts and observations but will represent an attempt, wherever possible, to interpret such findings in terms of the more fundamental chemical and physical alterations produced in the protein molecule under the influence of heat.

II. PROCESSES INVOLVING THE APPLICATION OF HEAT

It is beyond the scope of this chapter to describe in detail the various commercial processes used to produce vegetable protein meals. A description of oilseed processing is given in Chapter 4. Additional information will be found in Part II of this book and in other more specialized treatises on soybeans (4), cottonseed (5), and other meals (6). Only a brief survey of the more commonly used processing steps will be given here to enable the reader to appreciate the extent to which the application of heat is involved during the preparation of various vegetable protein meals.

1. Oil-Bearing Seeds

a. Operations Preceding the Removal of Oil

Several preliminary steps are necessary before oil-bearing seeds are suitable for the removal of the oil. Soybeans are subjected to cleaning, cracking, and dehulling, and cottonseeds are likewise cleaned, delinted, and decorticated. In addition, cottonseed meats are usually rolled in order to reduce the meats to a physical state which will promote the most efficient contact with the hot, humid vapor encountered in the subsequent cooking process. The operations which have thus far been mentioned do not involve the generation of sufficient heat to cause any appreciable effect on the properties of the protein.

Prior to extraction of the oil from soybeans in the screw press, soybean grits are dried in a steam tube to 2.5 to 3.0% moisture content at a maximum temperature of 140° to 160°. The cracked soybeans are then subjected to a further increase in temperature to about 170° to 180° in order to reduce the moisture content to a level of 2.0 to 2.5% prior to the meal's entering the screw press.*

Soybeans intended for solvent extraction are first converted into flakes in order to increase the surface exposed to the action of the solvent. To accomplish this the cracked beans are dried to a moisture content of 8 to 11% by heating to 60° to 70°. While the flakes are still hot they are passed through flaking rolls. Some processors steam the cracked beans prior to flaking in order to increase the plasticity and decrease the friability of the particles after flaking.

Cottonseed meats are cooked prior to hydraulic extraction for several reasons (5): to increase the fluidity of the oil, to coagulate the proteinaceous material, and to provide a satisfactory physical consistency for cake compression. Incidental to these effects, but of great importance from the nutritive standpoint (for non-ruminants), are the changes in pigment glands and gossypol induced by cooking. The cottonseed meats are cooked in stack cookers consisting of three to six compartments or in jacketed screw conveyors. This

* D. W. Crane, V. D. Anderson Co., Cleveland, Ohio, personal communication.

may be a continuous process in which the meats pass either from the top of the cooker to the bottom or through the conveyor. The process may also be operated batchwise in which the meats are cooked for a definite period of time in each compartment or kettle. The temperature of the meats in the top kettle is kept at 70° to 80° and is progressively increased as they pass through the cooker, leaving the bottom kettle at a temperature of about 110°. It is desirable to have flaked cottonseed meats of 11 to 12% moisture content in the top kettle, and the final moisture content after the completion of the cooking process should be 4.0 to 5.5%. In the screw-press process, the cottonseed meats are generally cooked at a somewhat higher temperature, 130° to 160°, which reduces the moisture to 2 to 3%.*

b. Mechanical Removal of the Oil

(1) *Hydraulic press.* After cooking, the oilseed meats are formed into cakes which are placed on press cloths and inserted into a hydraulic press. The usual hydraulic press can accommodate fifteen of such formed cakes. The rate of application of pressure varies considerably from one mill to another, but the final pressure is about 2000 p.s.i. applied over a period of about 30 minutes. The temperature of the cake inside the hydraulic press rarely exceeds that of the meats from the cooker.† Carter (6a) found that the press cake temperature varied from 55° to 130° from the top of the press to the bottom, the degree of variation also depending on the time of pressing, the temperature of the meats entering the press, and the temperature of the surrounding atmosphere.

(2) *Screw press.* Oilseeds, either whole, cracked, or flaked, are subjected to the crushing and shearing action of heavy screws which liberates the oil. This process is accompanied by the frictional generation of heat, but exact figures are difficult to quote, since conditions vary widely, depending on the equipment employed. Temperatures as high as 150°, however, have been recorded in the barrel of the press used for processing soybeans (4). In the screw-pressing of cottonseed meal, maximum temperatures of 130° to 170° have been recorded in the cage of the screw press (7). Because of the high temperatures involved in the screw-pressing operation, it is necessary to cool the cake immediately afterward by adding a small amount of water or by exposure to a draft of cool air. (See also the discussion on processing of safflower, Chapter 22.)

c. Solvent Extraction of the Oil

Extraction of the oil may be accomplished in a variety of extractors with commercial hexane being most commonly used as the solvent. With this solvent, extraction is carried out at the relatively low temperature of about 50° (6).

In the process combining screw-pressing and solvent extraction, the oilseed meats are cooked at temperatures and screw-pressed at pressures lower than in the usual screw-pressing operation, thus leaving a greater amount of residual oil in the cake. Subsequent extraction with commercial hexane reduces the oil content to less than 1% (8).

* D. W. Crane, V. D. Anderson Co., Cleveland, Ohio, personal communication.

† P. A. Williams, Southern Cotton Oil Co., New Orleans, La., personal communication.

d. Operations Subsequent to the Removal of the Oil

Cakes from which the oil has been extracted by mechanical means are ground and screened without any further treatment involving heat. In the case of solvent-extracted meals, however, it becomes necessary to remove the residual solvent. This is accomplished in a dryer or "desolventizer" at a temperature of about 98° over a period of 10 minutes. The direct application of steam for 5 to 10 minutes is sometimes employed to effect the removal of the last traces of the solvent. (See Chapter 10 for a description of a process of desolventization which involves much less heat.) Even if the soybeans have not been subjected to solvent extraction, as in the case of full-fat soybean flour, it becomes necessary to "debitter" the product by exposure to live steam.

Since considerably less heat is used in the solvent-extraction process, additional heat must be applied in the case of soybean meals if maximum nutritive value is to be obtained. This is accomplished in a toaster of which there are several types including vertical stack cookers, rotary tubular cookers, and pressure cookers. It is desirable to maintain an excess of moisture in the meal during toasting in order to increase the transfer of heat and produce a meal having a golden color. A temperature of 120° to 125° over a period of 20 to 30 minutes has been generally found to be most favorable for producing meals with the most desirable nutritive properties. Grinding and screening to produce a meal of uniform granulation complete the process.

2. Cereal Products

Many of the breakfast food cereals now on the market are examples of vegetable proteins which have been subjected to rather severe heat treatment during the course of processing. The manufacture of flaked cereals such as corn, bran, rice, or wheat flakes involves several stages during which heat is applied (9). The degerminated grains or "grits" are first cooked in an atmosphere of live steam for 2 to 2½ hours. After drying and tempering, the cooked grits are passed through flaking machines and then placed in toasting ovens. During toasting, the temperature, time, and moisture content are controlled to give the final product those characteristic qualities which appeal to the consumer.

Manufacture of puffed cereals involves somewhat more drastic heat treatment. The grits or doughs are enclosed in pressure chambers, sometimes called "puffing guns," where they are heated to temperatures as high as 300°. The pressure is suddenly released by opening the gun, thus causing the expanded water vapor and other gases to expand the product to several times its original volume. A final toasting operation reduces the moisture content, yielding a dry, crisp product.

3. Dehydrated Foods and Feed Components

The preservation of foods by dehydration is one of the oldest processes known to man, and interest in this method of preserving foods has been revived from time to time, particularly during emergencies

such as that engendered by World War II. It is generally agreed that the heat commonly employed for the drying of foods has a negligible effect on the nutritive value of the major food components. The adverse effect on nutritive value is confined largely to the water-soluble vitamins which are leached out during blanching or destroyed during storage (9a). The latter includes carotene and ascorbic acid, which are readily oxidized; riboflavin, which is destroyed by light; and thiamine, which is inactivated by sulfur dioxide. For these reasons, it does not seem profitable to describe here the methods used for dehydrating foods. For such details the reader is referred to Chapter 25 and to articles written by Mrak and Mackinney (9a) and by Gaver (10).

A number of feed components of vegetable origin such as corn, alfalfa, fermentation products, and yeast are prepared by drying. Information concerning the effect of heat on the nutritive value of such products is very meager, although one would expect the same considerations as indicated above to apply equally well here. In certain instances, however, it has been shown that excessive drying temperatures can adversely affect the nutritive value of corn and alfalfa. The improved stability of carotene in artificially dried alfalfa is an example of an instance whereby drying may actually improve the nutritive properties of a vegetable meal.

III. EFFECT OF HEAT ON THE NUTRITIVE VALUE

The term "nutritive value" will be used here in its broadest sense and will refer to the ability of a protein-containing food to promote the growth and well-being of an animal. This liberal definition is necessitated by the fact that in many instances the observed biological effects are not attributable to the effect of heat on the major protein constituents of the food but rather to the thermal inactivation of minor components which are capable of eliciting adverse physiological responses.

Purposefully omitted from consideration here will be the vast amount of information dealing with the thermal inactivation of those vitamins which are known to be susceptible to destruction by heat, light, and oxygen. This decision is prompted only by the necessity to conserve space and is justified perhaps by the fact that, from a practical point of view, vegetable protein meals are used primarily as a source of protein rather than micronutrients. Alfalfa leaf meal as a source of carotene and vitamins E and K, and dried yeast as a source of the B vitamins and other growth factors, may be considered as exceptions to this general statement. (See also the discussion in Chapters 16 and 17, on reduction in thiamine content through heat.)

It would be well to emphasize that much of the fundamental work relating to the effect of heat on the nutritive value of proteins has been done under laboratory conditions which are much more amenable to precise control of variables than commercial processes. The difficulty which sometimes attends the translation of laboratory results into actual operating conditions should be borne in mind in evaluating the significance of the experimental work which will be described below.

1. Soybean Oil Meal

a. Beneficial Effects

(1) *General observations with respect to growth.* Since soybean oil meal constitutes the largest single source of vegetable protein for livestock feeding, it is not surprising that a considerable amount of research has been directed toward a study of the factors which influence the nutritive value of this protein. These factors are discussed here as they pertain to heat effects and are discussed in Chapter 14, as they affect the utilization of the meal.

Shortly after the soybean was introduced into this country as a commercial crop, Osborne and Mendel (11), in a study of its potential value as a source of protein for animals, observed that soybeans would not support the growth of rats unless they had been cooked for 3 hours on a steam bath. The literature now abounds with innumerable reports confirming the superiority of heat-processed soybean oil meal, not only for rats (12-18) but also for mice (19), chicks (16, 20-23), turkey poults (24), swine (25-27), and human beings (28).

In general, these studies have shown that the degree of improvement in nutritive value effected by heat treatment is dependent on temperature, duration of heating, and moisture conditions. Commercially, these factors come into play during processing and exert an effect which is reflected in the nutritive quality of the final product. The data of Hayward *et al.* (13) and Wilgus *et al.* (20) (Table I) illustrate the extent to which the nutritive value of soybean protein may be improved, depending on the conditions of processing. In the laboratory, maximum nutritive value is obtained by proper adjustment of temperature, pressure, moisture content, and duration of heating. The curves in Fig. 1 show the effect of these variables on the nutritive value of ground soybeans as measured with rats (29). Two additional points are evident from these data, namely, the ineffectiveness of dry heat, and the marked impairment in nutritive value which accompanies excessive heat treatment. The latter effect will be considered in more detail later.

(2) *Nitrogen and sulfur metabolism.* In accordance with the general belief that the biological value of a protein is largely determined

TABLE I
EFFECT OF COMMERCIAL PROCESSING VARIABLES ON THE NUTRITIVE VALUE
OF SOYBEAN OIL MEAL^a

Screw-pressed meals					
Conditioner		Screw press		Protein efficiency ^{b,c} (PE)	Relative PE ^{c,d}
Temperature (°C.)	Time (min.)	Temperature (°C.)	Time (min.)		
Raw soybeans				0.51	38
90	13	105	2.0	0.58	47
100-112	13	112-130	2.5	1.15	80
100-112	13	140-150	2.5	1.45	84
Hydraulic-pressed meals					
Cooker		Hydraulic press		Protein efficiency ^{b,c} (PE)	Relative PE ^{c,d}
Temperature (°C.)	Time (min.)	Temperature (°C.)	Time (min.)		
Raw soybeans				0.51	60
82	90	65-75	50-60	0.82	80
105	90	65-75	50-60	1.14	88
121	90	68-80	50-60	1.15	82
Solvent-extracted meals					
Preliminary heating		Extractor	Desolventizer		Relative PE ^{c,d}
Tempera- ature (°C.)	Time (min.)	Tempera- ture (°C.)	Tempera- ture (°C.)	Time (min.)	
Raw soybeans				0.31	57
60	10	45	98	15	92

^a See also Chapter 14.

^b Grams gain in weight per gram of protein consumed.

^c Rat data taken from J. W. Hayward, H. Steenboch, and G. Bohstedt, *J. Nutrition* **11**, 219 (1936). Chick data taken from H. S. Wilgus, Jr., L. C. Norris, and G. F. Heuser, *Ind. Eng. Chem.* **28**, 586 (1936).

^d (PE of test diet/PE of casein diet) × 100.

by its amino acid content (30, 31), studies have been undertaken to determine if supplementation of the unheated protein with various amino acids would achieve the same effect as heating. It has thus been well established that the addition of methionine or cystine to unheated soybean meal improves protein utilization to essentially the same extent as proper heating (16, 23, 32–37). Amino acid analyses, however, revealed that the methionine content was substantially unchanged by the amount of heat necessary to produce maximum nutritive value (18,

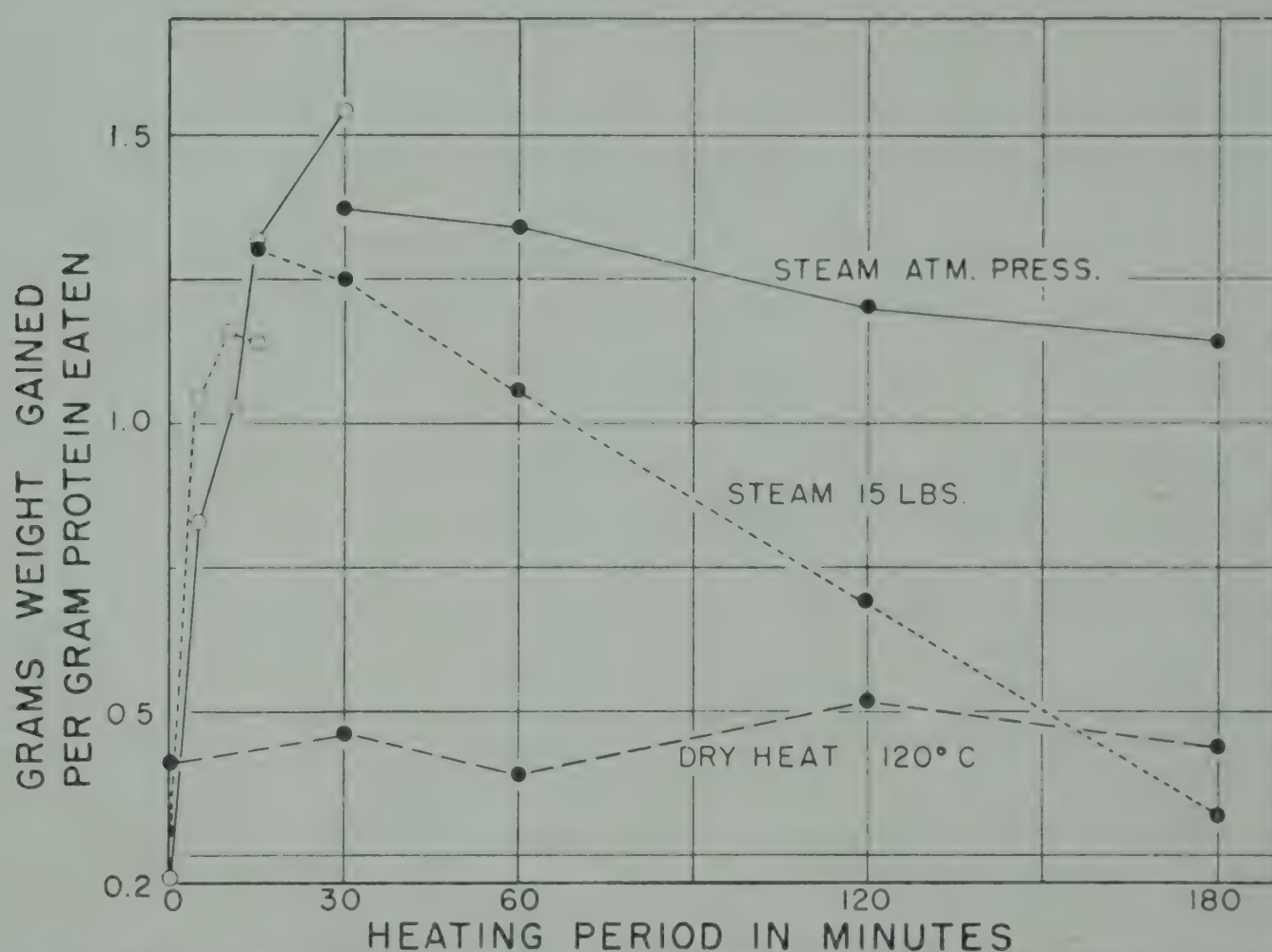


FIG. 1. The effect of type and extent of heat treatment on nutritional value of soybean protein (29). ●, experiment 1; ○, experiment 2. Test period, 42 days; 12 rats per group; 80 g. average initial weight. (Reproduced through the courtesy of *Food Technol.*)

32, 38–41). Although heated soybean meal was somewhat more digestible than the unheated meal in experiments conducted with rats,* the difference was too small to account for the marked differences in biological value (13, 17, 18, 44). In fact, the absorption of nitrogen (44, 45), sulfur (45, 46), or methionine itself (18, 47) from the digestive tract of the rat was essentially the same for both the raw and heated protein. A comparison of the absorption of nitrogen from the terminal 20% of the small intestine, however, revealed that over twice as much

* In contrast to the results obtained with rats, in chicks the digestibility of the protein and the absorption of methionine are significantly less in the unheated meal (23, 39, 42, 43).

nitrogen was absorbed from the heated protein as from the raw (44). Failure to confirm this observation has been reported (48). Regardless of the site of the absorption of the bulk of the protein, the retention of nitrogen and sulfur from raw soybean protein is nevertheless significantly less than from the heated protein (45). From these observations, summarized in Table II (49, 50), it would appear that the lower

TABLE II
OBSERVATIONS IN THE RAT REGARDING THE METABOLISM OF NITROGEN AND SULFUR
FROM RAW AND HEATED SOYBEAN MEALS

Type of measurement	Raw soybean meal	Heated soybean meal	References
A. Nutritive value			
1. Biological value	41	51	13
	53	71	18
	49	67	17
2. Protein efficiency	1.4	2.6	49
B. Nitrogen metabolism			
1. Per cent N absorbed	75 ^a	79 ^a	45
	77 ^a	81 ^a	44
	33 ^b	79 ^b	44
	67 ^b	75 ^b	48
2. Per cent N retained	18	33	45
C. Sulfur metabolism			
1. Per cent sulfur absorbed	59	64	45
	80	76	46
2. Per cent methionine absorbed	51	53	18
3. Per cent sulfur retained	12	30	45
	13	26	46
4. Per cent methionine available for growth	44	71	50

^a As measured from fecal analyses.

^b As measured from the terminal 20% of the small intestines.

nutritive value of unheated soybean meal was not the result of incomplete digestion of the protein, but rather that the methionine was absorbed in a form, or possibly at a site, from which it cannot be effectively utilized for growth.

(3) *Trypsin inhibitor*. Melnick *et al.* (18), on the basis of observations on the *in vitro* release of amino acids from soybean protein by the enzyme pancreatin, suggested that the methionine of raw soybean protein was liberated more slowly by the proteolytic enzymes of the intestines than the other essential amino acids so that it was not available for mutual supplementation of the latter. This concept was sup-

ported by the discovery (51, 52), purification (53, 54), and characterization (55-61) of a heat-labile protein in soybeans which inhibits the proteolytic activity of trypsin. Active antitryptic preparations from unheated soybeans have been shown to retard the growth of rats (49, 62, 63), mice (19), and chicks (63, 64). Because the protein efficiency of partially heated soybean flours increased in proportion to the destruction of the trypsin inhibitor, Westfall and Hauge (19) concluded that the trypsin inhibitor was the major cause of the poor utilization of the protein in raw soybeans. Almquist and Merritt (65, 66) found that the growth inhibition of chicks was almost fully developed when as little as one-fourth of the dietary protein was furnished in the form of the raw meal. Crude (65, 66) or crystalline (67) trypsin was capable of reversing this growth inhibition.

The foregoing evidence strongly suggests that the poor growth-promoting qualities of raw soybeans can be attributed to an inhibition of intestinal proteolysis. According to the hypothesis of Melnick *et al.* (18), one would expect the trypsin inhibitor to retard specifically the enzymatic release of methionine so that it is not available in time to supplement the other essential amino acids released earlier. Subsequent reports (38, 40, 68, 69), however, do not support such a conclusion, since heat treatment was found to increase the enzymatic release of other amino acids to the same proportionate extent as methionine. Almquist and Merritt (70, 71) have questioned the necessity of postulating a specific interference with the enzymatic release of methionine to explain the methionine deficiency provoked by raw soybean meal. They believe that the action of the inhibitor is a general interference with digestion* so that a substantial amount of the most limiting amino acid of soybean protein, methionine (32) is excreted unabsorbed, thus precipitating a methionine deficiency. In confirmation of this concept, these authors have shown with chicks that the addition of the trypsin inhibitor in the form of raw soybean meal to rations containing marginal levels of lysine, arginine, isoleucine, or tryptophan caused these rations to become markedly deficient in these particular amino acids (70, 71).

This explanation may be valid for chicks where, in contrast to rats, more methionine is excreted from raw soybean meal than from heated meal (see footnote on p. 86). There is good evidence, however, that the growth-retarding effect of raw soybean meal on rats and mice may not be due to its antiproteolytic activity. Thus, active antitryptic prepara-

* This idea is supported by the observation that other amino acids such as tryptophan (72) are excreted by the chick to a larger extent from the raw meal than from the heated protein.

tions have been shown to retard the growth of rats (49, 73) and mice (74) when incorporated into rations containing predigested protein. A related observation is the report by Borchers and Ackerson (75) that the growth inhibition produced by raw soybean meal could be counteracted by a fraction of crude trypsin which was devoid of proteolytic activity.

(4) *Soybean hemagglutinin*. The results of a study of the effect of supplemental methionine on the nutritive value of diets containing crude concentrates of the trypsin inhibitor led Liener *et al.* (49) to suggest that the trypsin inhibitor exerts its deleterious effect on growth by a combination of two mechanisms: one, an impairment in the availability of methionine due to the antiproteolytic activity of the inhibitor, and the other, an effect which was unrelated to an inhibition of proteolysis. In order to dissociate these two effects, the crude trypsin inhibitor preparation was injected into rats so as to circumvent intestinal proteolysis (76). Single doses of the crude inhibitor proved to be toxic at an LD₅₀ level of 200 mg./kg. body weight, whereas subtoxic doses inhibited growth in proportion to the frequency of injection. Crystalline soybean trypsin inhibitor was non-toxic at an equivalent level of antitryptic activity. The toxic component of the crude inhibitor preparation was subsequently isolated (77) and characterized as a hemagglutinin because of its ability to agglutinate red blood cells (78–79).^{*} Feeding trials with rats indicated that the soybean hemagglutinin was responsible for about one-half of the growth inhibition obtained with raw soybean meal (81).

(5) *The effect of vitamin B₁₂ and antibiotics*. It has been reported (82–84) that vitamin B₁₂ is capable of improving the nutritive properties of raw soybean meal in much the same manner as heating. This effect is similar to that produced by methionine supplementation and may in fact be due to the methionine-sparing action which vitamin B₁₂ is known to display (85). Aureomycin has likewise been shown to improve the nutritive value of unheated soybean meal to a greater extent than the heated protein (86), an effect which was attributed to a sparing action of the antibiotic for dietary methionine (87). A combination of aureomycin and streptomycin was particularly effective in improving the nutritive value of raw soybean oil meal but had little effect on the already improved nutritive properties of the heated meal (88).

^{*} This substance was originally given the name of "soyin." It was unfortunately overlooked that Laufer *et al.* (80) had previously used this term in referring to a proteolytic enzyme system in soybeans which does not appear to have been further characterized. To avoid confusion, the name "soyin" will not be used here but the substance will be hereafter referred to as the soybean hemagglutinin.

(6) *Saponin*. Potter and Kummerow (89) have described the isolation of a saponin from soybeans (soyasapogenol B) which they claim inhibited chick growth, although no growth data were presented in this preliminary communication. It was suggested that the beneficial effect of heat on soybeans was due to the hydrolysis of the saponin to the non-toxic sapogenin. Although cholesterol has been shown to counteract the growth-depressing effect of alfalfa saponins on chicks (90), cholesterol did not improve the nutritive value of unheated soybean meal.*

(7) *Other growth inhibitors*. In a preliminary report Weaver (91) has described a substance from raw soybeans which inhibits the growth of rats and *Escherichia coli*. From a description of its properties it appears to be different from any of the growth inhibitors known to be present in raw soybeans. Its most characteristic properties are its dialyzability and solubility in 80% ethyl alcohol. It is insoluble in 95% ethyl alcohol, ether, and petroleum ether, and is not retained by ion-exchange resins.

(8) *Other antinutritional factors*. In addition to factors which adversely affect the growth performance of experimental animals, raw soybeans are also known to contain a number of heat-labile substances which exert deleterious physiological effects not revealed by growth or metabolism studies.

Unheated soybean meal has been shown to cause a marked enlargement of the thyroid in rats (92) and chicks (93) which could be counteracted by iodine and partially eliminated by subjecting the soybean meal to heat.

Bouthilet *et al.* (43) have postulated the existence of a heat-labile fraction in raw soybeans which has a marked diuretic effect on chicks.

Balloun and Johnson (94) found that the blood-clotting time of chicks was significantly increased by feeding diets containing unheated soybean meal. It is quite likely that the factor responsible for this effect is the trypsin inhibitor, since the latter has been found to inhibit the coagulation of blood *in vitro* (95). The hypertrophy of the pancreas of chicks fed raw soybean meal observed by Chernick *et al.* (96) can perhaps be explained as a compensatory response to the trypsin inhibitor.

The inclusion of 30% or more of ground raw soybeans in the diet of dairy calves lowers the levels of vitamin A and carotene in the plasma (97). Although lipoxidase might logically be suspected as the causative agent because of its ability to destroy carotene, roasting soybeans at 100° for 30 minutes did not prove beneficial (98). Since

* I. E. Liener, unpublished observation.

lipoxidase measurements were not made, it is not known whether this amount of heat treatment was sufficient to inactivate the enzyme.

b. Adverse Effects of Overheating

It has been recognized for some time that excessive heating during commercial processing or under laboratory conditions (see Fig. 1) may adversely affect the nutritive value of soybean protein (13, 15, 20, 23–26, 99, 100). The damage which the protein suffers from excessive heat apparently involves changes in the lysine and methionine, since both of these amino acids are required to restore its maximum nutritive

TABLE III
PROTECTIVE EFFECT OF WATER ON THE NUTRITIVE VALUE OF OVERHEATED
SOYBEAN OIL MEAL FOR CHICKS^a

Meal	Time autoclaved at 15 lb.	Water added (%)	Average weight at 3 weeks	
			Practical ration ^b (g.)	Semisynthetic ration ^c (g.)
1	4 min.	0	203.5	177.5
2	30 min.	0	208.6	174.5
3	4 hr.	0	135.0	72.0
4	4 hr.	25	165.9	101.8
5	4 hr.	50	161.3	126.0
6	4 hr.	100	162.6	139.0

^a R. Renner, D. R. Clandinin, and A. R. Robblee, *Poultry Sci.* **32**, 582 (1953).

^b Soybean oil meal, 30%; corn, 39.5%; wheat bran, 10%; wheat shorts, 10%; alfalfa meal, 5%; vitamins and minerals to 100%.

^c Soybean oil meal, 38.5%; dextrin, 49.7%; corn oil, 5.0%; vitamins and minerals, to 100%.

value (24, 29, 35, 99, 100). Although moisture appears to be necessary for the improvement of the nutritive value of soybean meal by heat (see Fig. 1), it is interesting to note that large amounts of water can partially prevent the damaging effects of excessive heat (101) (Table III).

Severe heat treatment to the point of nutritive impairment leads to a destruction of a number of amino acids, particularly lysine, arginine, tryptophan, and cystine (36, 38, 39, 102). *In vitro* and *in vivo* digestibility studies are in accord that the digestibility of soybean protein is considerably depressed by overheating (23, 39, 42). The obvious manifestation of this decreased digestibility is a retardation in the rate at which all the amino acids are released from the protein by suitable *in*

vitro proteolytic systems (38, 103). According to the view of Almquist (104), since methionine is the limiting amino acid of soybean protein, an impairment in digestion leading to an excretion of methionine serves to accentuate a deficiency in this amino acid. The critical need for methionine is further intensified by the fact that about one-third of the cystine, which normally spares methionine to a certain extent, is destroyed by excessive heating of soybean meal (36). The unavailability of lysine is particularly acute not only because of the poor digestibility of the protein but also because almost one-half the lysine may be destroyed (102). These facts therefore provide a ready explanation for the observed methionine and lysine deficiencies of overheated soybean meal.

2. Other Legumes

a. General Properties

As for soybean oil meal, the nutritive value of many other legumes is also improved by proper heat treatment. In Table IV are summarized the observations recorded in the literature (105–155) relating to the effect of heat on nutritive value, and the presence of heat-labile factors which inhibit trypsin or agglutinate red blood cells.

The widespread distribution of a trypsin inhibitor in legumes provides the most likely explanation for the observation that heating increases the *in vitro* digestibility of a number of legumes (127, 156). Jaffé (157) observed that those legumes which had the highest trypsin inhibitor activity were also those in which the digestibility, as measured *in vivo*, was most improved by cooking. It has been generally noted that the supplementation of uncooked legumes with cystine (108) or methionine (34, 111, 139) markedly improves their nutritive value. This effect is readily understandable in view of the fact that methionine is the limiting amino acid of most leguminous proteins (158), and hence the action of a proteolytic inhibitor would be expected to accentuate this deficiency in accordance with the views expressed by Almquist (104).

Several lines of evidence, however, suggest that the poor nutritive value of some uncooked legumes cannot be satisfactorily explained by this concept. Klose *et al.* (159) found that lima bean fractions possessing high *in vitro* antitryptic activity also inhibited the growth of rats fed acid-hydrolyzed casein. Inspection of the data in Table IV further reveals that there is no obvious correlation between the effect of heat on the nutritive value of various legumes and the presence or absence of a trypsin inhibitor, a conclusion which has also been reached by Borchers and Ackerson (124) and Jaffé (160).

TABLE IV

EFFECT OF HEAT ON THE NUTRITIVE VALUE OF LEGUMES, AND THE PRESENCE OR ABSENCE OF TRYPSIN INHIBITORS AND HEMAGGLUTININS^a

Common name	Botanical name	Nutritive value	Trypsin inhibitor	Hemagglutinin
Navy bean	<i>Phaseolus vulgaris</i>	+ (105-111)	+ (112, 113)	+ (114-116)
Kidney bean				
Pinto bean				
Lima bean	<i>Phaseolus lunatus</i>	+ (105, 106, 111, 117)	+ (112, 118, 119)	+ (120, 121)
Adsuki bean	<i>Phaseolus angularis</i>	- (122)	?	?
Mung bean	<i>Phaseolus aureus</i>	± (123-125)	+ (112)	+ (116)
Velvet bean	<i>Stizolobium deeringianum</i>	+ (124, 126, 127)	+ (112)	?
Guar bean	<i>Cyamopsis psoraloides</i>	± (124)	- (112)	?
Horse bean	<i>Vicia faba</i>	+ (124)	- (112)	+ (116, 128)
Common vetch	<i>Vicia sativa</i>	± (124)	- (112)	+ (116, 129)
Hyacinth bean	<i>Dolichos lablab</i>	+ (130)	+ (130, 131)	+ (130)
Castor bean ^b	<i>Ricinus communis</i>	+ (132, 133)	?	+ (134)
Lentil	<i>Lens esculenta</i>	+ (124, 125, 135)	+ (113)	+ (129)
		± (107, 136)	- (112)	
Southern pea	<i>Vigna sinensis</i>	+ (124, 137-139)	+ (112)	?
Cowpea		± (111)		
Blackeyed pea				
Jack bean	<i>Canavalia ensiformis</i>	+ (124, 140)	- (112)	+ (141)
Partridge pea	<i>Chamaecrista fasciculata</i>	+ (124)	+ (112)	?
Field pea	<i>Pisum sativum</i>	- (105, 111, 142-145)	- (112)	+ (129, 146)
		± (137)		
Sweet pea	<i>Lathyrus odoratus</i>	± (147)	?	+ (116)
Lespedeza	<i>Lespedeza stipulacea</i>	± (124)	+ (112)	?
Garbanzo	<i>Cicer arietinum</i>	+ (148)	+ (149)	- (116)
Bengal gram		± (125)		
Peanut	<i>Arachis hypogaea</i>	+ (150)	+ (112, 155)	?
		± (124, 151, 152)		
		- (150, 152-154)		

^a + indicates that the nutritive value is improved by heat, or positive evidence for the presence of a trypsin inhibitor or hemagglutinin; - indicates that the nutritive value is adversely affected by heat, or the absence of a trypsin inhibitor or hemagglutinin; ± indicates the nutritive value is unaffected by heat; ? indicates that information is not available. Italic numbers in parentheses refer to appropriate references.

^b The castor bean is a member of the Spurge family and is not a legume. (See Chapter 31.)

Although hemagglutinins are found in practically all legumes, with the exception of the castor bean and soybean hemagglutinins, little is known about the nutritional significance of this group of substances. Ricin has been long known to be responsible for the extreme toxicity of castor bean meal (161), and the soybean hemagglutinin is believed to be responsible for about one-half of the growth-inhibiting properties of unheated soybeans (81). The role which hemagglutinins play in the nutritional qualities of other legumes would appear to merit further study.

The possible presence of other growth inhibitors in legumes should not be overlooked. Jaffé (130, 162), for instance, has postulated the existence of a heat-labile toxic factor in kidney beans and claims to have isolated a fraction which is markedly toxic at a dietary level of 0.05% (162).

b. Peanut Meal

It will be noted in Table IV that the nutritive value of peanut protein may be improved, unaffected, or depressed, depending on the severity of the heat treatment applied. Cama and Morton (150) attribute the beneficial effect of moderate heat treatment to the destruction of a trypsin inhibitor (112, 155). Little or no effect on the nutritive value of peanut protein is caused by solvent or hydraulic-press extraction of the oil (151), boiling in water for 30 minutes (152), or autoclaving at 15 pounds pressure for 30 minutes (124). Peanuts subjected to commercial roasting (152, 155) or screw-pressing (150, 154), however, suffer some damage in nutritive value. A significant difference in digestibility in rats between raw and roasted peanuts was observed by Mitchell's group (153). The beneficial effect of lysine when added to diets containing peanut protein damaged by roasting (152) indicates that lysine is one of the amino acids rendered unavailable by severe heat treatment. Although experimental evidence is lacking, one would predict that severe heating would also accentuate the requirement for methionine, since this amino acid is limiting in peanut protein (30). (See also Chapter 16.)

c. Castor Bean Meal

Reference has already been made to the toxic properties of castor bean meal which renders this meal practically useless as a source of protein for animal feeds. Castor bean meal prepared by solvent extraction is particularly toxic (132, 133), whereas meals which have been prepared at the more elevated temperatures of screw-pressing have considerably reduced toxicity (133). Solvent-extracted meals can be

rendered completely non-toxic by autoclaving for at least 15 minutes (132, 133), but such meals are still inferior in growth-promoting qualities when compared to an equivalent level of protein provided by casein (133). Amino acid analyses show the protein of castor bean to be deficient in tryptophan, methionine, and lysine (133). The presence of heat-stable growth inhibitors and thermal damage to the protein are other factors which may contribute to the poor nutritive qualities of detoxified castor bean meal. (See also Chapter 31.)

d. Peas

Contrary to the beneficial effects of heat observed with most legumes, the nutritive value of the field pea is damaged by baking, canning, or autoclaving (105, 111, 142-145). This impairment in nutritive value is amenable to correction by supplementation with cystine or methionine (143, 145). Evans and St. John (163) found that autoclaving peas for 1 hour at 130° destroyed 24% of the cystine and 25% of the lysine and, in addition, decreased the *in vitro* digestibility of the protein. Schneider and Miller (145), however, were unable to demonstrate any difference in *in vivo* digestibility between raw and cooked peas. Since methionine is the limiting amino acid of pea protein (143), it would appear that the destruction of cystine is the primary factor leading to the methionine deficiency of cooked peas.

3. Cottonseed Meal

Since cottonseed meal was one of the first vegetable protein meals to be fed to livestock in the United States, numerous investigations were undertaken at the turn of the century relative to the nutritive value of cottonseed protein. In many instances it was noted that commercial cottonseed meals produced untoward effects on farm animals which were attributed to dietary deficiencies of one sort or another. The significance of these early studies is difficult to evaluate, since they were done at a time when the importance of vitamins and other dietary essentials was not clearly established. At any rate, the feeding of cottonseed meal to certain farm animals was looked on as a practice which was not always to be recommended.

Once the importance of these factors was understood, cottonseed meal gained acceptance as a cattle feed. Since 1940 there has been an extensive program of research on cottonseed meal directed primarily toward extending its use in feeds for non-ruminants. As a result, a sizeable proportion of cottonseed meal is being used for feeding poultry and swine as well. Processing methods, their effect on composition, and their relationship to the usefulness of the meal in various feeding situa-

tions are presented in detail in Chapter 17. The discussion which follows is intended to call attention to the effect of heat on the quality of the protein and on the reactions involving gossypol. A discussion of the "gossypol problem" is also included in the chapter on cottonseed meal.

a. Free Gossypol Theory

Although the presence of gossypol in cottonseed was reported as early as 1886 by Longmore (164) and isolated by Marchlewski in 1899 (165), its relationship to the toxic effects of cottonseed was not appreciated until 1915. In that year Withers and Carruth (166-168), and somewhat later Schwartz and Alsberg (169-170), presented evidence which clearly established gossypol as the substance primarily responsible for the toxic effects of the cottonseed kernel.* It had already been recognized that commercial cottonseed meals were generally less toxic than the original kernel. The explanation for this came from the studies of Osborne and Mendel (177) and Gallup (178-180), who pointed out the importance of heat and moisture in reducing the toxicity of cottonseed, and the reports by Carruth (181) and Sherwood (182) that the free gossypol (extractable with ether or aqueous acetone) decreased on cooking or commercial processing. These observations led to the belief that free gossypol was solely responsible for the toxicity of cottonseed meal, and the effect of heat and moisture was to liberate the gossypol from the pigment glands, causing it to react with the protein of the seed to form a non-toxic "bound" form of gossypol (183-184).

b. Protein Damage and Gossypol Inactivation

Notwithstanding this attractive hypothesis, Gallup (185) was unable to obtain a satisfactory correlation between the nutritional value of various cottonseed meals and their free gossypol content, a finding which led him to conclude that other factors must influence the nutritional properties of the protein. Olcott and Fontaine (186) demonstrated that cottonseed meal, which had been rendered free of gossypol by extraction with ether in the absence of heat, underwent a loss in nutritive value in proportion to the severity of heat treatment (see Table V). Similar results have been recently reported by Condon *et al.* (187) using cottonseed meal which had been degossypolized by extraction with methyl ethyl ketone (188). It is thus evident that the effect of heat on the nutritive quality of cottonseed meal is twofold: (1) inactivation of gossypol, and (2) damage to the protein.

* That the toxicity of the cottonseed pigment glands is due solely to gossypol has been questioned by Eagle and his associates (171-176a).

Recognition of this dual nature of the effect of heat on cottonseed meal stimulated research dealing with a re-examination of the nutritional importance of free gossypol under conditions not involving heat injury to the protein. This line of research was facilitated by the availability of meals produced under known and controlled processing conditions. Among them were meals of low free gossypol content but subjected to varying degrees of severity of heat treatment, from solvent-extracted meals of minimum heat damage to some screw-pressed meals which had been damaged extensively. There were also made available cottonseed pigment glands and pure gossypol for experiments with small animals (189, 190). Out of this work came the realization that

TABLE V
EFFECT OF AUTOCLAVING ON THE NUTRITIVE VALUE OF ETHER-EXTRACTED
COTTONSEED MEAL^a

Treatment	Protein efficiency
Unheated	1.99
Autoclaved, 30 min. at 120°	1.58
Autoclaved, 60 min. at 120°	1.08
Autoclaved, 120 min. at 120°	0.28

^a H. S. Olcott and T. D. Fontaine, *J. Nutrition* **22**, 123 (1946).

there exists a level of free gossypol in the meal at which there is no interference with growth of chicks. This level has been set generally at 0.04% or thereabouts, although Couch *et al.* (191) and others consider that the level can be higher, even up to 0.1%. (See Chapter 17.)

The establishment of a level of free gossypol which does not interfere with growth made possible a study of the specific effect of heat damage to the protein. This effect is illustrated by the data of Table VI taken from the studies of Milligan and Bird (192) and Horn *et al.* (193). The free gossypol content of all these meals is well below the growth-inhibiting level for chicks, yet the conditions of processing cause marked variations in their nutritive value. (It should also be borne in mind that impaired hatchability (194) and discoloration of the egg yolk (195) may result from levels of free gossypol which would not be expected to affect the growth of chicks.)

c. Possible Significance of Bound Gossypol

A number of other studies (196–198) have likewise indicated little or no correlation of growth response with low levels of free gossypol. One aspect of these studies has been the negative correlation observed between nutritive value and *bound* gossypol. The possible physiological significance of bound gossypol would therefore appear to merit further consideration. Although

TABLE VI
EFFECT OF COMMERCIAL PROCESSING VARIABLES ON THE NUTRITIVE VALUE
OF COTTONSEED MEAL

Maximum temperature in cooker (°C.)	Cooking time (min.)	Free gossypol in meal ^a (%)	Feed efficiency ^{a,b} (chicks)	Relative protein efficiency ^{c,d} (rats)
<i>Screw-pressed meals</i>				
Standard ^e		0.08		100
81	20	0.01	0.278	86
93	70	0.01	0.314	80
93	20	0.01	0.264	90
112	20	0.01	0.266	73
128	40	0.02	0.256	68
128	20	0.02	0.261	69
137	100	0.03	0.161	22
<i>Hydraulic-pressed meals</i>				
116	72	0.05	0.250	68
110	36	0.10	0.266	80

^a Taken from the data of J. L. Milligan and H. R. Bird, *Poultry Sci.* **30**, 651 (1951).

^b Total gain in weight per total feed consumption in a practical starter ration containing 39% cottonseed meal.

^c Taken from the data of M. J. Horn, A. E. Blum, M. Womack, and C. E. F. Gersdorff, *J. Nutrition* **48**, 231 (1952).

^d Protein efficiency of standard taken as 100.

^e Gland-free meal prepared by the gland flotation process; H. L. E. Vix, J. J. Spadaro, R. D. Westbrook, A. J. Crovetto, E. F. Pollard, and E. A. Gastrock, *J. Am. Oil Chemists' Soc.* **24**, 228 (1947).

bound gossypol has never been isolated as a chemical entity, it is generally considered to be a gossypol-protein complex in which gossypol has reacted with the ϵ -amino groups of protein-bound lysine (184, 199, 200). On the basis of theoretical considerations to be discussed later, protein-bound lysine, in which the ϵ -amino group is substituted, would render the adjacent peptide bond (containing the carbonyl moiety of lysine) unavailable to splitting by trypsin. It is interesting to note how nicely this type of reaction explains a number of observations recorded in the literature:

1. The ability of gossypol to inhibit the *in vitro* digestibility of cottonseed globulin (201).
2. The resistance of bound gossypol to digestion by the chick (199).
3. The decreased availability of lysine when gossypol was added to solvent-extracted cottonseed meal fed to rats (202).
4. The increased fecal excretion of nitrogen when gossypol was added to the diet of cats (170).

All these observations suggest that the formation of bound gossypol, if not the cause, is at least associated with the adverse effect of heat on the *in vivo*

digestibility (203) and *in vitro* enzymatic release of amino acids (204). Among the amino acids thus affected, lysine becomes the most critical, since it is the limiting amino acid of cottonseed protein (30). Although the lysine of cottonseed protein may be destroyed to a slight extent by excessive autoclaving (202), commercial meals differing widely in nutritive value showed little difference in lysine content. Decreased digestibility leading to the unavailability of lysine would seem therefore, to be the primary factor accounting for the beneficial effect of adding lysine to heat-damaged cottonseed meals (186, 197). Further discussion of bound gossypol and of destruction of lysine will be found in Chapter 17.

4. Cereal Proteins

The first extensive study relating to the effect of heat on the nutritive value of cereal proteins was made by Morgan (205, 206). The toasting of wheat, rice, or corn at 150° to 200° for 30 to 45 minutes was found to reduce the growth-promoting quality of the protein. Subsequent feeding studies on both animals (154, 207–210) and human beings (211, 212) have amply confirmed Morgan's earlier observations concerning the deleterious effect of heat on certain cereal proteins.* Severe heat treatment such as that employed in the manufacture of certain puffed cereals is particularly damaging to the protein quality of wheat (206, 209, 211, 212), oats (207), rice (206), and cereal mixtures (207, 208, 212). In most instances it has been difficult to detect a significant depression in digestibility as a consequence of heat treatment except where the gun explosion process has been employed (208).

Kon and Markuze (215) found that the crust of the bread, which receives more severe heat treatment during baking than the crumb, has a lower nutritive value than the crumb. Greaves and Morgan (216) attribute this difference in nutritive value to the decreased digestibility of the crust. Another factor contributing to the poorer nutritive value of the crust is the destruction of lysine during baking to the extent of about 15% (217).

Hathaway *et al.* (210) conducted a study to determine the effect of drying ear corn from an initial moisture content of 27% or more to a final value of 14%. Heated air under forced draft at temperatures ranging from 50° to 110° was employed. Drying temperatures in excess of 60° were found to depress the nutritive value of the corn for rats to a significant extent.

* An apparent exception to this general observation is the report by Beaudoin *et al.* (213) that the cooking of whole wheat under laboratory conditions to simulate shredded wheat produces a significant improvement in nutritive value. Sure (214), however, found that rats on commercial shredded wheat gained only 81% of the weight of animals receiving whole wheat flour.

A good illustration of the manner in which the nutritive value of a cereal product may be affected by the severity of the heat treatment is given by the data of Block and his associates (218) shown in Table VII. Although lysine restored the nutritive value of the most severely damaged cake sample, there was no indication that the lysine had been destroyed. Mitchell and Block (208) had similarly observed no evidence of lysine destruction in an oat-rye mixture whose nutritive value had been seriously reduced by the gun explosion process.

A report by Peters *et al.* (219) serves to clarify many aspects of this problem. A study was made of the effect of various methods of processing oat protein on amino acid composition and the *in vitro* release of

TABLE VII
EFFECT OF HEAT ON THE NUTRITIVE VALUE OF A CEREAL PRODUCT^a

Sample	Treatment	Protein efficiency
1	Raw cake mix ^b	3.4
2	Sample 1, baked, and air-dried on radiator for 24 hours	2.4
3	Sample 1, baked, and oven-dried at 60° overnight	1.5
4	Sample 1, baked, and toasted at 100° to 130° for 1 hour	0.7
5	Sample 4 plus 0.63% lysine	3.2

^a R. J. Block, P. R. Cannon, R. W. Wissler, C. H. Steffee, Jr., R. L. Straube, L. E. Frazier, and R. L. Woolridge, *Arch. Biochem.* **10**, 295 (1946).

^b Contains the following ingredients: flour, 51%; sucrose, 19.7%; egg white, 11.5%; lactalbumin, 7.6%; oil, 5.2%; yeast, 3.0%; molasses, 1.1%; and salt, 0.9%.

amino acids by tryptic digestion. These data were correlated with the growth performance of rats fed diets containing the processed proteins. Amino acid analyses of acid and enzyme hydrolyzates of the raw and heat-processed oat protein revealed that the only essential amino acid to be affected to any extent was lysine. The effect of heat treatment in determining the manner in which the lysine became unavailable is shown in Table VIII.

Increasing the severity of heat treatment caused a progressive decrease in the ease with which lysine could be released from the protein by enzyme action. More severe heat treatment such as toasting and gun explosion actually caused an appreciable destruction of lysine as evidenced by the amount of lysine that could be recovered from acid hydrolyzates. The addition of lysine restored the nutritive value of the oat protein damaged by the toasting process. Another experiment, conducted to compare the relative amounts of lysine excreted by animals fed unprocessed and exploded oats, revealed that 22.5% of the lysine

derived from the exploded oats was excreted in the feces compared to 7.2% of the lysine from raw oat protein.

If these results can be extended to other cereal proteins, it can be generalized that heated cereal proteins are more refractory to the action of proteolytic enzymes than their unheated counterparts. A delay in the release of lysine in the intestinal tract could account for an impairment in nutritive value according to the hypothesis of Melnick *et al.*

TABLE VIII
EFFECT OF VARIOUS PROCESSING METHODS ON THE AVAILABILITY OF LYSINE
FROM OAT PROTEIN^a

Sample	Lysine content		
	Acid hydrolysis	Enzyme hydrolysis ^b	Protein efficiency
Raw oat dough ^c	100 ^d	100 ^d	100 ^d
Cooked dough ^e	100	89	99
Toasted flakes ^f	82	63	72
Puffed oats ^g	68	44	23
Toasted flakes plus 0.188% lysine	124	112	98

^a F. N. Peters, R. Carroll, W. R. Bunting, and G. W. Hensley, "Project Report, Committee on Food Research." Quartermaster Food and Container Institute for the Armed Forces, Chicago, Ill., 1947.

^b Digested with trypsin for 72 hours.

^c Oat flour, glucose, water, and flavoring.

^d Relative values calculated on the basis of 100% for raw oat dough.

^e Raw oat dough cooked for 1 hour under 15 pounds pressure.

^f Cooked dough toasted at 200° for 2 minutes.

^g Raw oat dough preheated for 5 minutes at 122° followed by cooking at 200 pounds pressure, 198° for 2 minutes.

(18) without necessarily affecting the over-all digestibility of the protein measured *in vivo*. With more severe heat treatment, however, the decrease in the rate with which lysine is released is apparently great enough to exceed the length of time that the protein is subjected to the action of the animals' proteolytic and absorptive mechanisms. Thus, the decreased digestibility of heat-processed cereal proteins observed by some investigators (208) contributes further to a lysine deficiency because of the excretion of a disproportionate fraction of lysine in the feces. Superimposed on the non-availability of lysine due to its resistance to enzymic release from the protein is the actual destruction of lysine which may occur if the heat treatment is sufficiently severe. Since lysine is the amino acid limiting the biological value of cereal

proteins, any factors contributing to its unavailability will greatly impair the nutritive quality of the protein.

5. Sunflower Seed Meal

Morrison *et al.* (220) have reported that screw-pressed sunflower seed meals processed at a maximum temperature of 115° supported better growth than meals processed at a maximum temperature of 130°. The latter meal was found to have lost 15% of its lysine and 7% of its arginine (221). No relationship was observed, however, between the nutritive value of these meals and the liberation of essential amino acids by enzymatic hydrolysis. Alexander and Hill (222) also noted that autoclaving or dry heating of solvent-extracted sunflower seed meal caused considerable destruction of lysine in proportion to the duration of heating. As might be expected, the nutritive value of these overheated meals could be markedly improved by supplementation with lysine. (See also Chapter 19.)

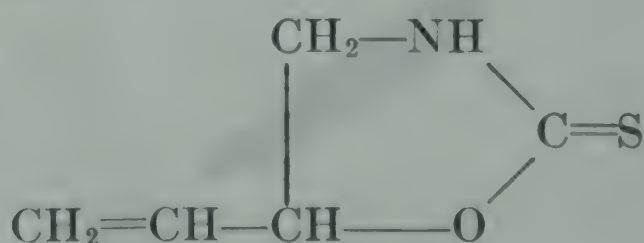
6. Linseed Meal

Linseed meal has been shown to depress the growth of chicks, an effect which can be prevented by extracting the meal with water (223), supplementing the diet with pyridoxine (224), or autoclaving the meal for 30 minutes at 15 pounds pressure (225). The observation that screw-processed linseed meal is superior to a meal extracted at low temperatures with ethylene dichloride (154) may be due to the destruction of this same growth inhibitor. The latter appears to be largely associated with the cotyledon fraction of the flaxseed (226).

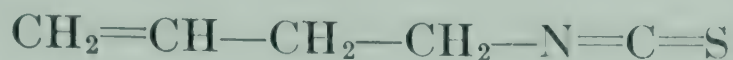
Flaxseed is also known to contain a cyanogenetic glycoside, *linamarin*, from which HCN is liberated by the enzyme linamarase (227). This enzyme is destroyed during expression of the oil by either the hydraulic or screw press methods so that the resulting meals contain non-toxic levels of HCN (228). (See also Chapter 21.)

7. Rapeseed Meal

The evidence for the presence of toxic factors in rapeseed meal has been comprehensively reviewed by Bell (229). As a member of the genus *Brassica*, rapeseed contains the goitrogenic factor L-5-vinyl-2-thioxazolidone (230, 231):



It is believed that the precursor of this substance in rapeseed is mustard oil, allylisothiocyanate:



which is naturally bound as a non-toxic glycoside called *gluconapin*. This glycoside can be hydrolyzed by the enzyme *myrosinase* to liberate mustard oil which subsequently becomes oxidized and cyclized to yield L-5-vinyl-2-thioxazolidone.

Fröhlich (231) has reported that dry heat, 130° for 24 hours, reduces the goitrogenic activity of rapeseed meal for chicks. It is not certain, however, whether this reduced goitrogenicity is due to the destruction of myrosinase or the goitrogenic principle. Higher temperatures, 150° for 5 hours, produced a non-toxic meal which had rather poor growth-promoting quality, probably as a consequence of heat injury to the protein. (See also Chapter 20.)

8. Mustard Seed Meal

Mustard seed also contains mustard oil in the form of the toxic glycoside *sinigrin* (232). *Myrosin* is the enzyme which releases mustard oil which in itself is relatively non-toxic. Mustard seed can be rendered safe for animal consumption by subjecting the screw-pressed cake to steaming for 1 to 2 hours, a treatment which destroys the glycoside and volatilizes the mustard oil (228).

9. Tung Meal

There have been conflicting reports regarding the toxicity of commercial tung meal, some workers (233–235) having found it to be unpalatable and toxic, whereas others (236, 237) have indicated it to be relatively innocuous. Davis *et al.* (238) and Emmel (239) have demonstrated that toxic commercial tung meals cannot be detoxified by heat alone. Emmel (239) found, however, that by extracting toxic meals with 95% ethyl alcohol for 6 to 8 hours and then autoclaving at 15 pounds pressure for 30 minutes in the presence of dilute acid he could obtain a product which was non-toxic and was capable of supporting good growth in chicks. Emmel concluded that the toxicity of tung meal was due to two principles: a saponin which was destroyed by hot acid hydrolysis, and a toxic substance which was heat-stable but soluble in ethyl alcohol. He was able to concentrate the second factor and show that it had toxic and growth-inhibiting properties toward chicks. It was also noted that the toxicity of the alcohol-soluble fraction was gradually lost on storage, which may explain some of the divergent reports in the literature concerning the toxicity of tung meal. Lee and

Watson (235) have confirmed the dual nature of the toxic constituents of tung meal and further conclude that detoxified tung meal is of low biological value and is not an effective supplement to the cereal proteins of a basal chick ration. Lysine appears to be the first limiting amino acid of detoxified tung meal (240). Further information on the toxic materials in tung meal is in Chapter 31.

10. Alfalfa Leaf Meal

Autoclaving is not an effective means of counteracting the growth-inhibiting properties of alfalfa leaf meal, presumably owing to its saponin content (90, 241). In fact, a report by Zimmerman (242) indicates that heat may exert a deleterious effect on the nutritive value of alfalfa leaf meal. This author found that air-drying alfalfa leaves at 170° for 60 minutes caused a significant reduction in digestibility and weight gain in rats.

Alfalfa is frequently employed in livestock rations as a rich source of carotene. The field-curing process, however, may lead to the destruction of 45 to 90% of the carotene by enzymatic oxidation or destruction by light (243). The stability of carotene may be considerably enhanced by employing artificial drying, an effect which has been attributed to the inactivation of carotene oxidase (lipoxidase) (244). (See also Chapter 25.)

11. Protein Isolates

Only a limited number of studies have been conducted on the effect of heat on proteins isolated from crude vegetable protein meals. It should be emphasized at the outset that the term "protein isolate" does not necessarily imply that such proteins are pure or homogeneous according to presently accepted criteria for protein purity (245). Since they do, however, in most instances represent the major protein components of the seed, it may be of interest to compare the effect of heat on the nutritive value of such preparations with similar studies on the crude meals already considered.

a. Soybean Protein

Although unheated soybean meal is an unsatisfactory source of protein for growth, Osborne and Mendel (246) found that *glycinin* was capable of supporting good growth in rats. De and Ganguly (247) reported that isolated soybean protein had a biological value superior to the raw meal and equivalent to autoclaved soybean meal. In view of our present knowledge, these observations provide additional evidence

that the poor nutritive value of unheated soybean meal is due to components other than the main protein constituents of the soybean.

The glycinin used by Osborne and Mendel referred to a globulin-like protein obtained by extracting soybeans with 10% NaCl, precipitating with saturated ammonium sulfate, redissolving in water, and finally reprecipitating by dialysis (248). This procedure was somewhat modified by De and Ganguly (247), who extracted the meal with 6% NaCl, dialyzed, and precipitated the protein at pH 4.2. Electrophoretic (249, 250) and sedimentation (251) studies have shown that such preparations are far from homogeneous.

b. Other Leguminous Proteins

Studies have been made of the effect of cooking on the nutritive value of the globulin-like proteins of the navy bean (108) and the velvet bean (252), designated as *phaseolin* and *stizolobin*, respectively. Cooking was found to improve the growth-promoting properties of these isolated proteins, particularly when supplemented with cystine. *In vitro* digestibility studies on stizolobin (127) indicated that cooking likewise enhanced the susceptibility of this protein to the combined action of pepsin and trypsin. Lüning and Bartels (110) found that the protein of the white bean, which they called *phasin*, inhibited the growth of mice unless heated.

Although distinctive names have been given to these various protein preparations, their mode of preparation would indicate them to be complex protein mixtures. Phaseolin (252) and phasin (110) were simply the protein which had been precipitated from aqueous extracts of the bean by dilution with water in the case of phaseolin or by adding alcohol in the case of phasin. Stizolobin was the protein precipitated by dialysis from a saline (10% NaCl) extract of the velvet bean, and the heated protein was the coagulum formed when the saline extract was boiled. Contamination with the trypsin inhibitor might account for the poor growth and the indigestibility of these unheated proteins *in vitro*. Lüning and Bartels (110) also refer to the marked hemagglutinating activity which their preparation of phasin exhibited.

Peanut protein was dispersed from raw peanuts, and solvent-extracted or hydraulic-pressed meals at pH 7 or 9 followed by precipitation at pH 4.5 to 4.6 (151). These protein isolates produced weight gains in chicks similar to that obtained with the original crude meal (151).

c. Cottonseed Protein

The ability of cottonseed globulin to sustain normal growth in rats was established by Osborne and Mendel (177). This is of course in marked contrast to the toxicity of the original cottonseed kernel.

Studies on the effect of heat on isolated cottonseed protein have not been reported.

d. Cereal Proteins

Wheat gluten appears to be the only cereal protein on which the effect of heat on nutritive value has been studied. Morgan (206) first demonstrated the deleterious effect of toasting on the nutritive value of gluten, and, more recently, Halevy and Guggenheim (253) have reported that gluten autoclaved in the presence of glucose undergoes a further impairment in nutritive value. In the latter instance, there was considerable destruction of lysine as well as a decrease in the extent to which lysine could be released by enzymatic hydrolysis. Addition of lysine quite logically improved the biological value of the heated gluten-protein mixture. Protein-carbohydrate interaction is considered in more detail in a later section.

e. Hemp Seed

The nutritive value of *edestin*, a crystalline protein from hemp seed, was considerably reduced by autoclaving for 5 hours at 15 pounds pressure (254). Supplementation with lysine was effective in restoring the growth of rats to the level obtained with the unheated protein.

IV. CHEMICAL AND PHYSICAL CHANGES INDUCED BY HEAT

1. Destruction and Inactivation of Amino Acids

a. General Observations

In the preceding section it was seen how studies relating to the effect of heat on the nutritive value of proteins have revealed that an impairment in nutritive value is frequently accompanied not only by the destruction of certain amino acids but also by an increased tendency of the protein to resist enzymatic digestion. For convenience in discussion these two effects will hereafter be referred to as the "destruction" and "inactivation" of amino acids. More specifically, destruction will be defined as the difference between the amounts of an amino acid which can be measured in the acid (or alkaline in the case of tryptophan) hydrolyzates of the raw and the heated proteins. Inactivation* will be defined as the difference between the amounts of an amino acid which can be measured in deproteinized hydrolyzates after subjecting the raw and the heated proteins to *in vitro* enzymatic digestion under stand-

* It is implied in this definition that suitable corrections will be made for the destruction of amino acids in the heated protein.

ardized conditions. For more details concerning methodology the reader is referred to the appropriate references where given.

That the destruction of amino acids in crude protein systems such as represented by soybean oil meal is not due to the effect of heat on protein alone was indicated by the work of Patton *et al.* (255). Soy globulin refluxed for 24 hours in water did not lead to the destruction of any essential amino acids, whereas refluxing the protein with 5% glucose destroyed lysine, arginine, and tryptophan—the same amino acids which are destroyed by overheating soybean oil meal (38). Since soybean oil meal is known to contain about 22% carbohydrate-like material (256), it was apparent that an interaction between protein and carbohydrate was most likely responsible for the destruction of these particular amino acids in the crude meal.

Evans and his associates (36, 102, 257–260) have made a detailed study of the role which carbohydrate plays in the destruction and inactivation of the amino acids of the protein in soybean meal. A summary of their results comparing the destruction and inactivation of amino acids in soybean meal, soybean protein, and soybean protein plus sucrose is depicted in Fig. 2.

From these results Evans has concluded that the *destruction* of amino acids under the experimental conditions employed involves a reaction of protein-bound amino acids with sucrose* to give a complex which is either resistant to acid hydrolysis or, what is more likely, cannot be utilized for growth by the assay organism. Functional nitrogen groups not attached in peptide linkages characterize the amino acids which are destroyed in this manner, namely, lysine, arginine, histidine, and tryptophan. The destruction of cystine in soybean oil meal is attributed to some other, still unknown, mechanism (36).

The *inactivation* of protein-bound amino acids likewise is believed to be due to a reaction with sucrose or with some other constituent of the protein. Most of the amino acids appear to be inactivated to a variable extent by reaction with sucrose, whereas only the dicarboxylic or basic amino acids such as aspartic acid, glutamic acid, lysine, arginine, and histidine are inactivated by reaction with other protein constituents.

b. The Browning Reaction

It has been known for some time that proteins and carbohydrates interact under a variety of conditions. As early as 1912 Maillard (261) had disclosed a reaction between amino acids and reducing sugars which

* It is quite likely that extensive inversion of sucrose had occurred under the conditions employed by Evans and his group. The reactive sugar would then be an acyclic aldose isomer in equilibrium with cyclic forms of glucose and fructose.

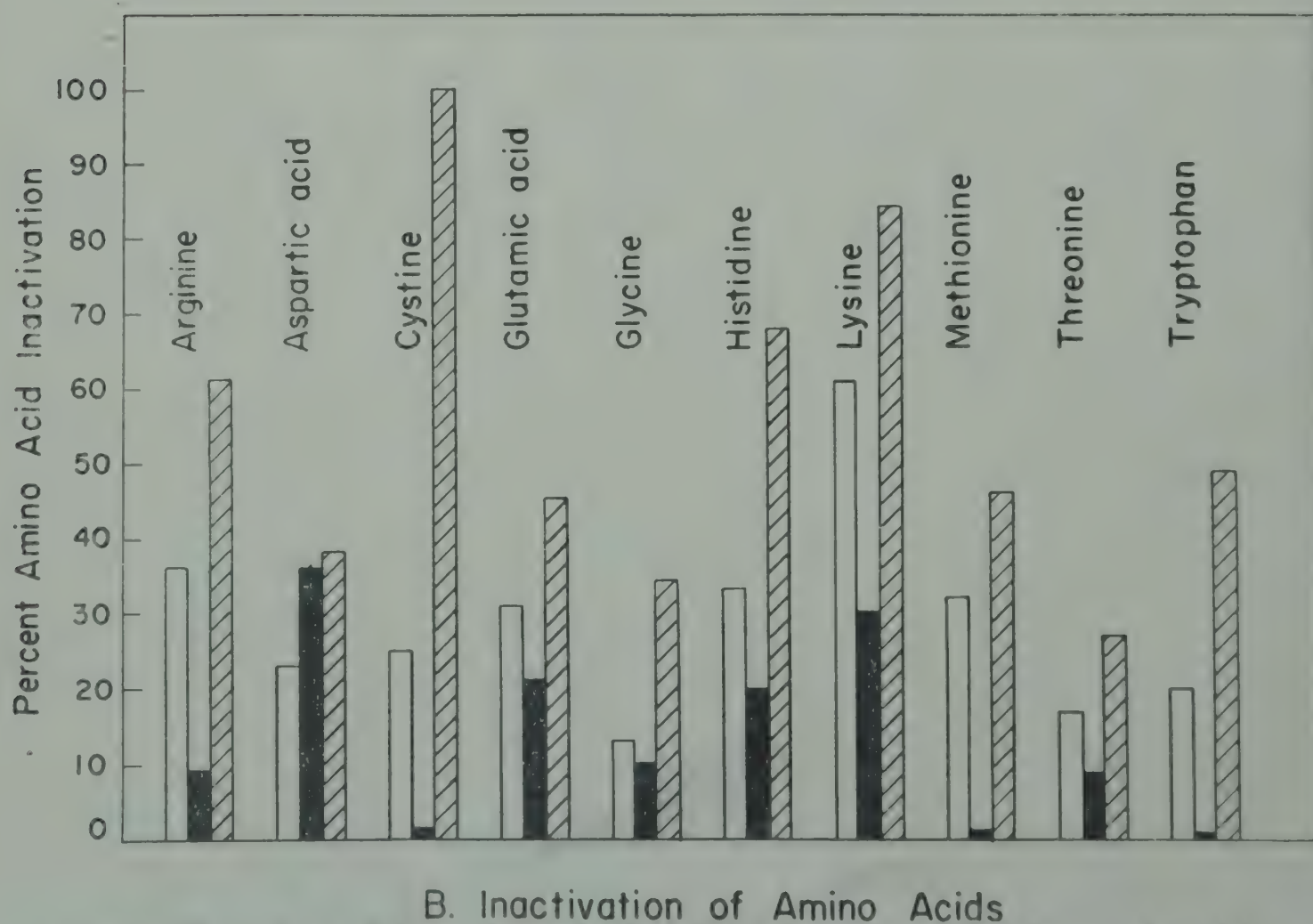
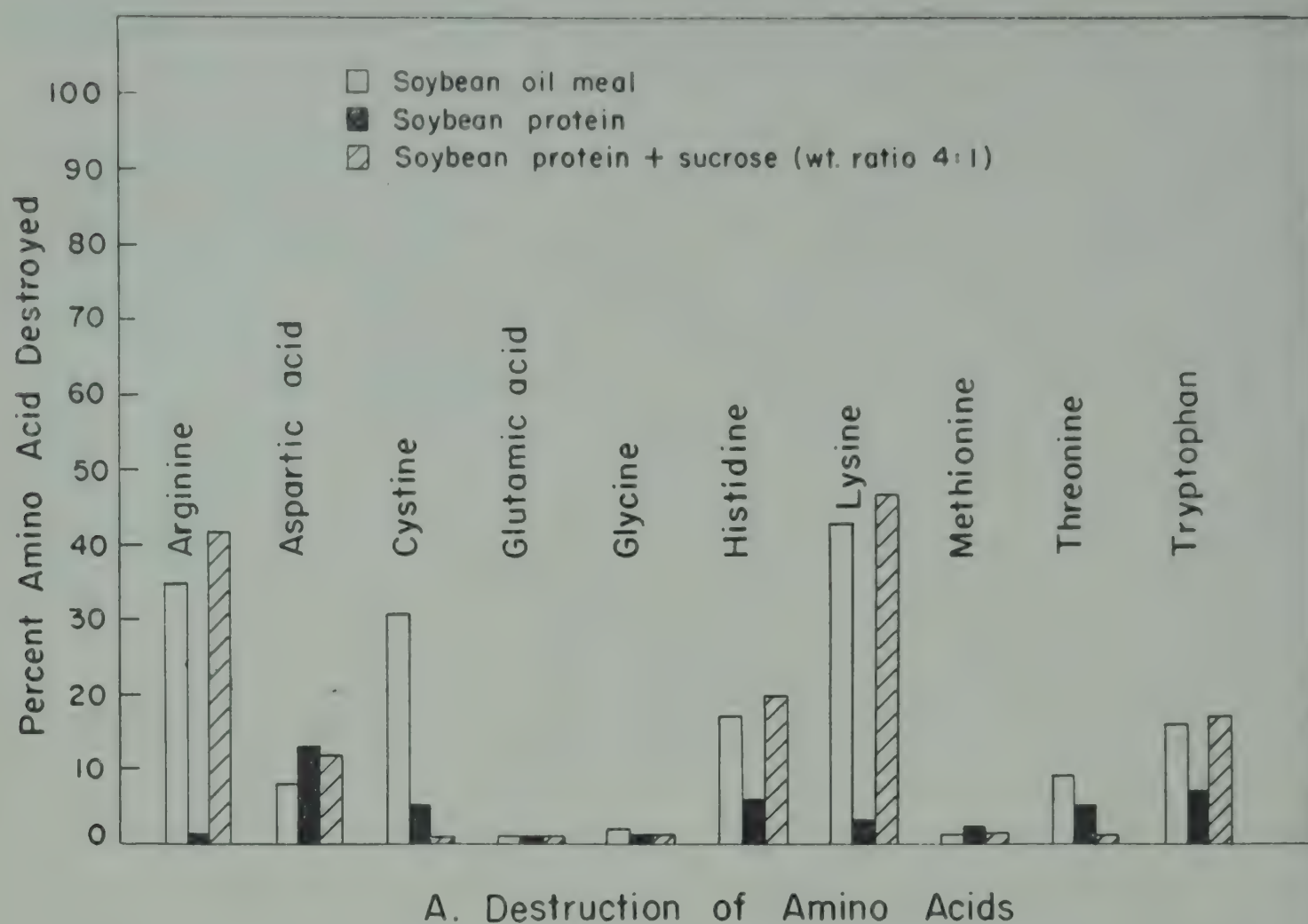


FIG. 2. The effect of heat (autoclaving for 4 hours) on the destruction (A) and inactivation (B) of amino acids in soybean oil meal, soybean protein, and soybean protein plus sucrose. These charts have been constructed from data contained in the papers of Evans and his associates (36, 102, 257-260).

was accompanied by a darkening of the reaction mixture. It is obviously beyond the scope of this review to consider in any detail the vast amount of research that has been conducted through the years on the so-called browning reaction (262, 263). Although the work that has been done with simple model systems has contributed much to our understanding of the mechanism of the browning reaction, primary consideration will be given here to those observations pertaining to protein-bound amino acids and carbohydrates.

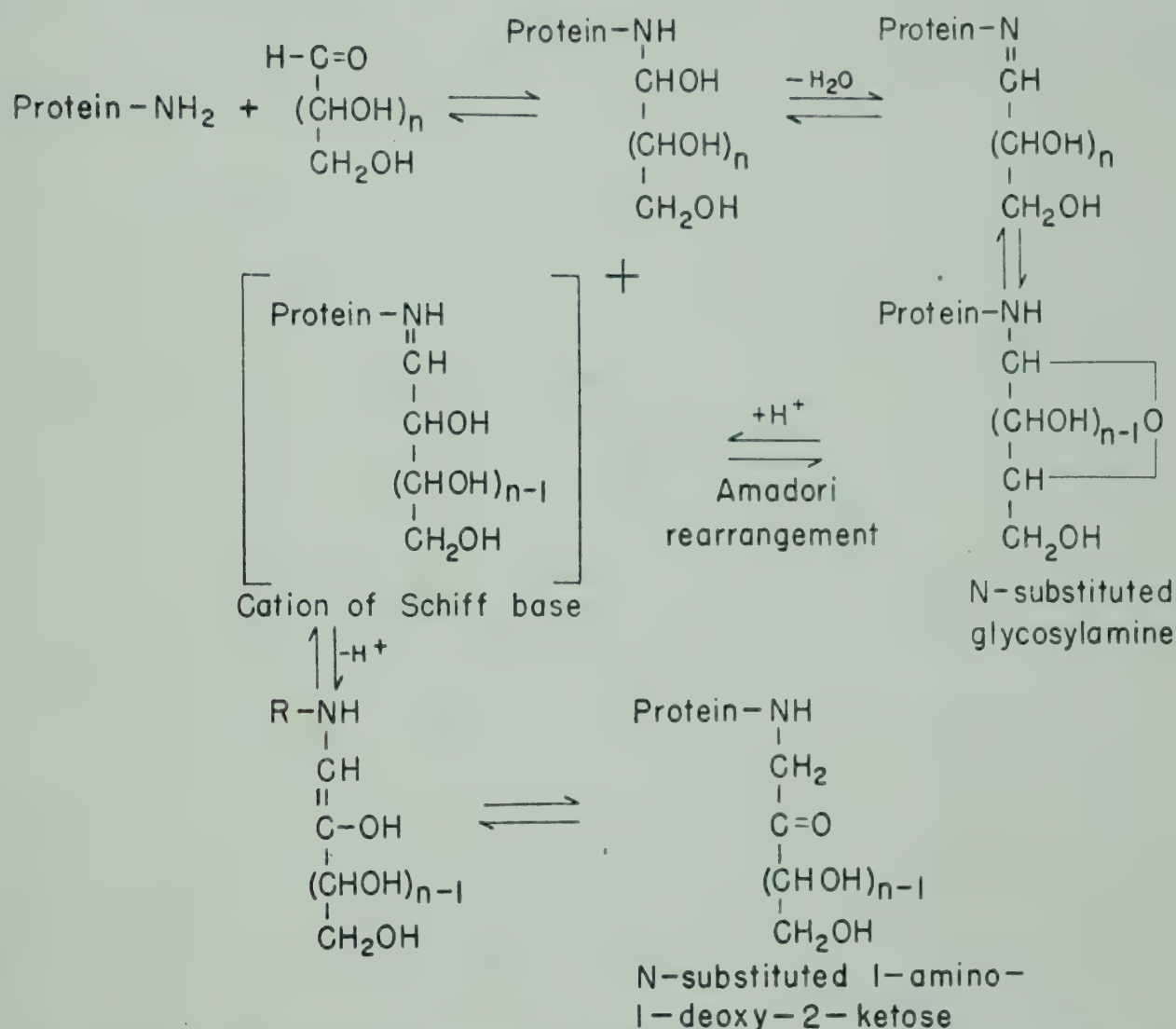


FIG. 3. Sequence of reactions between free amino groups of protein and reducing sugars.

Much of our knowledge regarding the chemical changes that ensue during the interaction of a protein with reducing sugars can be credited to the fine work of Lea and Hannan (264-267), who employed casein and glucose as model reactants. The most rapid chemical change observed in their system was a loss of free amino groups, mainly the ϵ -amino groups of lysine, and the formation of bound glucose in a molar ratio of 1:1. At this stage of the reaction the complex was still colorless and lysine could be quantitatively recovered by acid hydrolysis. From various lines of evidence, reviewed by Hodge (263), it is believed that the reaction of the free amino groups of the protein with glucose proceeds according to the sequence of reactions shown in Fig. 3.

The classical studies of Bergmann and his group (268-270) have established that the enzyme trypsin splits a protein molecule at those peptide bonds containing the carbonyl moiety of lysine or arginine provided the ϵ -amino group of lysine or the guanido group of arginine is unsubstituted. Substitution of these groups via the series of reactions shown in Fig. 3 would produce a peptide bond which would be resistant to the action of trypsin. At this stage of the protein-sugar reaction a situation obtains where, although lysine could be recovered by acid hydrolysis, the protein would no longer be susceptible to cleavage by trypsin. Experimental observations regarding the general inactivation of amino acids in crude protein systems are in accord with this theoretical concept.

As the interaction of casein and glucose proceeded beyond the 1:1 stage (267), the mixture darkened, and lysine, arginine, and histidine could no longer be recovered by acid hydrolysis. With the exception of tryptophan these are the same amino acids which have been observed to be most sensitive to heat destruction in vegetable protein meals. Failure to recover these amino acids on acid hydrolysis could be due to a reaction of the nitrogen groups not involved in a peptide linkage with sugars in a fashion analogous to that shown in Fig. 3. Instead of the reaction stopping with the formation of the N-substituted 1-amino-1-deoxy-2-ketose, however, the latter would undergo a further breakdown into various intermediates leading ultimately to the formation of brown nitrogenous polymers known as *melanoidins* (263).

It has been recently reported that the reaction between certain proteins or model polypeptides and glucose can occur in the absence of visible browning and proceeds most rapidly in the dry state (271, 272). In the latter instance, the *in vitro* digestibility of the protein or polypeptide complex was considerably reduced compared to a system in which the reactants were dissolved in water. In this connection, it is of interest to recall the observation of Renner *et al.* (101) (Table III) concerning the protective action of water on soybean oil meal exposed to excessive heat treatment.

As indicated previously, the inactivation of amino acids which does not depend on the presence of sugars is confined to the dicarboxylic or basic amino acids. The most likely explanation for this observation is the formation of linkages between the free carboxyl groups of glutamic or aspartic acids and the free amino groups of lysine and arginine or the imidazole group of histidine. The linkage so formed, although of the peptide type, would not fulfill the substrate specificity requirements necessary for cleavage by the appropriate proteolytic enzymes, but would be split by acid digestion.

2. Protein Solubility

Denaturation of protein has been defined as "any non-proteolytic modification of the unique structure of a native protein giving rise to definite changes in chemical, physical, and biological properties" (273). Lessened solubility is perhaps the most common physical evidence of a protein which has undergone denaturation by heat. The solubility of a protein frequently reflects the amount of heat treatment received during processing and is therefore of interest from the nutritional point of view. In addition the solubility of vegetable proteins in various solvents determines their usefulness for many industrial purposes. (See also Chapter 10.)

a. Soybean Oil Meal

Much of the work relating to the effect of heat on the solubility of soybean protein has been done under laboratory conditions where precise control of temperature, time, moisture, and pressure is possible. Using solubility in water as the criterion for denaturation, Beckel *et al.* (274) studied the effects of temperature and moisture on the rate of denaturation of the protein of solvent-extracted soybean oil meal. The curves in Fig. 4 clearly show how denaturation is dependent not only on the temperature employed but also on the amount of moisture in the atmosphere. Even intense dry heat, however, when applied over a long period of time (152° for 18 hours) will eventually cause soybean protein to become totally insoluble (275).

Evans and St. John (276) studied the effect of heat on the solubility of soybean protein fractions obtained by extracting successively with water, 5% potassium chloride, 70% ethyl alcohol, and 0.2% potassium hydroxide. At 121° there was a progressive increase with time of heating, up to 30 minutes, in the amount of nitrogen which could be extracted with 0.2% KOH (glutelin fraction) accompanied by a concomitant decrease in nitrogen solubility in water and 5% KCl. Applying the same technique to trichloroethylene-extracted soybean oil meal, Burnet and Arnold (277) reported a similar trend over a wide range of laboratory processing conditions.

Recognizing the difficulty of translating laboratory conditions into commercial operating procedures, Belter and Smith (278) studied the solubility in water of the protein of samples removed at various stages of the solvent-extraction process. The data pertaining to samples taken from five different plants employing widely different processing conditions are shown in Table II, Chapter 10. These data clearly indicate that the major portion of the denaturation occurs in the final stages of deodorizing and toasting. It is important to note that the meals produced for livestock feeding had lower values for dispersible nitrogen than the meals intended for specialty products. The desirability of attaining high protein solubility in soybean meals prepared for industrial usage will be considered in a later section.

b. Cottonseed Meal

On the basis of studies by Olcott and Fontaine (279), solubility in 3% NaCl (0.5 M NaCl) has been frequently used as the criterion for the de-

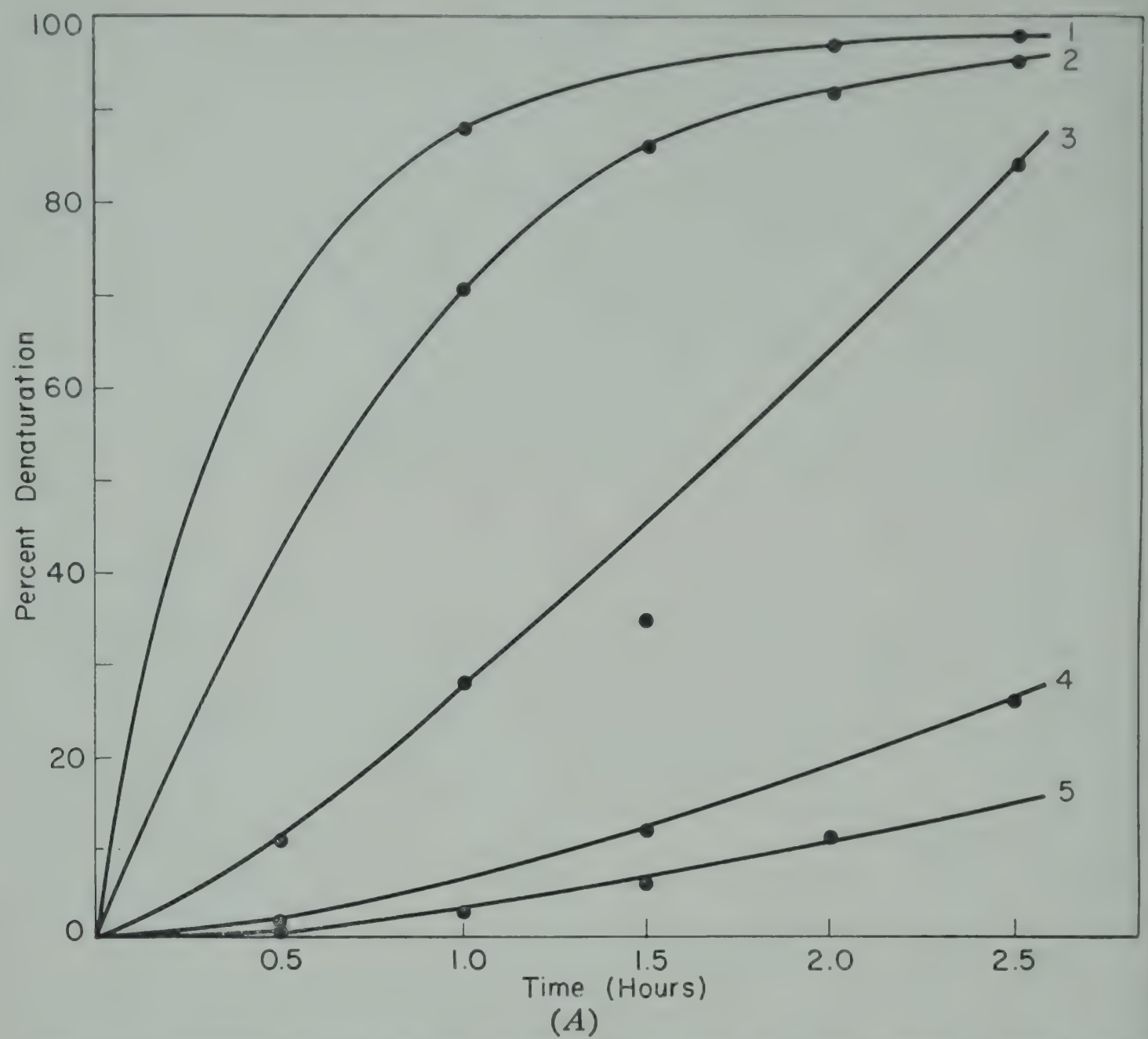


FIG. 4. Denaturation of solvent-extracted soybean oil meal when heated under various conditions (274). (A) Curves 1 to 5; (B) curves 6 to 11. (Reproduced through the courtesy of *Ind. Eng. Chem.*)

Curve	Temperature (°C.)	Relative humidity (%)
1	127	100
2	100	100
3	90	100
4	80	100
5	60	100
6	80	18
7	100	0
8	100	18
9	100	50
10	120	0
11	120	18

naturation of cottonseed protein. Olcott and Fontaine observed that the solubility of cottonseed protein in 3% NaCl was considerably reduced by autoclaving and varied widely among different commercial meals. Thus, the solubility of the protein from fifteen commercial cottonseed meals ranged from 9.6 to 46.7% compared to a value of 75 to 80% for ether-extracted meals. Screw-pressed meals generally undergo a greater reduction in protein solubility than meals produced by hydraulic pressing, the nitrogen solubility of screw- and hydraulic-pressed meals averaging about 12.3% and 31.7%,

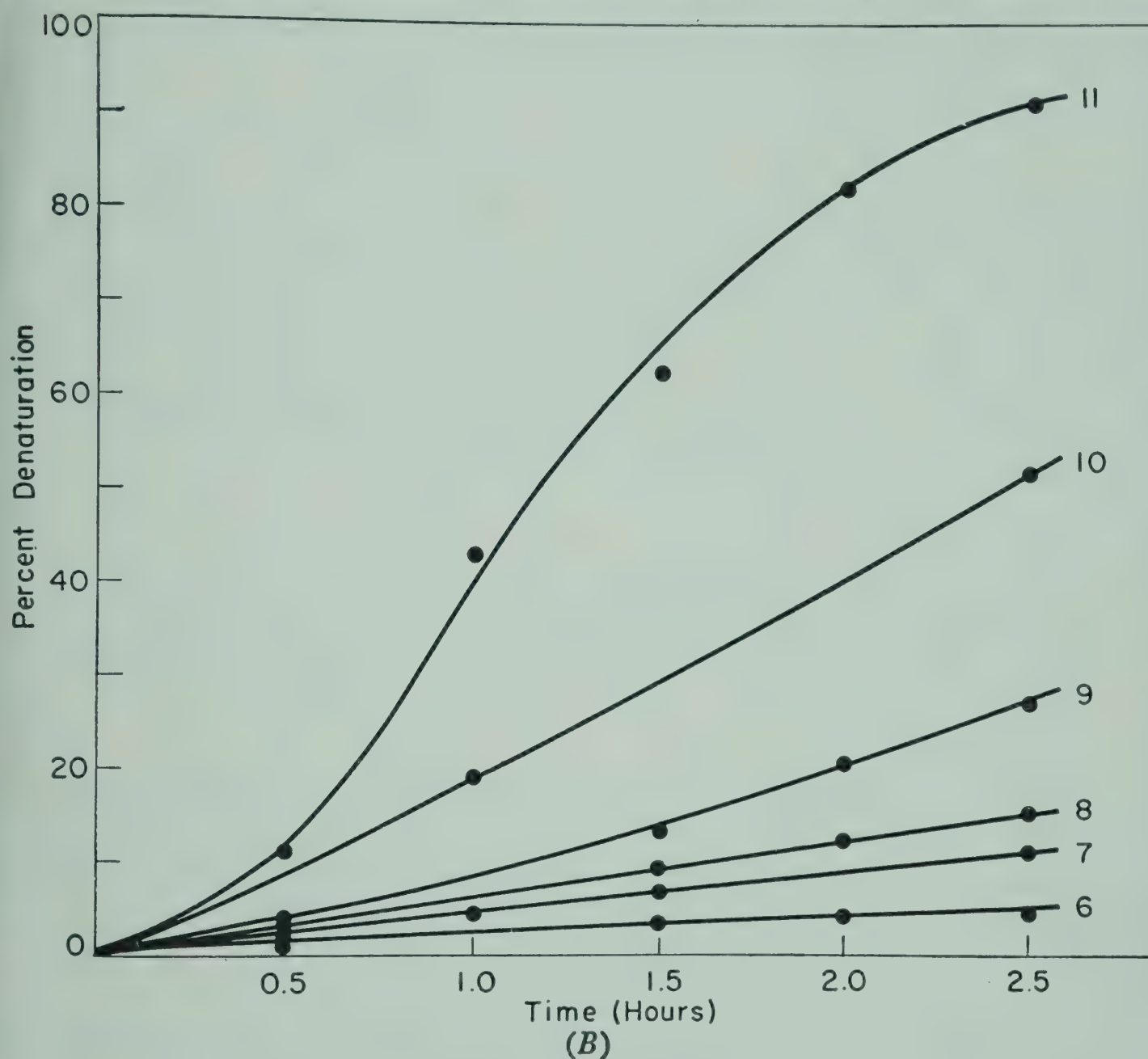


FIG. 4 (Continued).

respectively (280). This difference may be attributed to the higher temperatures employed during cooking which precedes screw-pressing, but particularly to the elevated temperatures developed in the screw press itself. The protein solubility of screw-pressed meals is also affected by the moisture content of the meats during the cooking phase. Thus, meals produced from meats which had been cooked at a moisture content of 7% had a higher protein solubility than meals whose meats had a moisture content of about 10% during cooking (281). The solubility of the protein from meals prepared by the prepress-solvent process, is somewhat greater than the protein from either the screw- or hydraulic-pressed meals (8).

Solubility of the protein in 0.02 N NaOH has also been used to measure the denaturation of cottonseed protein (187, 197, 198), and this value seems

to parallel more closely the nutritive value of cottonseed meals than solubility in dilute saline as originally proposed by Olcott and Fontaine (279). Further discussion of solubility of cottonseed protein will be found in Chapter 17.

c. Peanut Meal

The solubility of peanut protein does not appear to be as readily affected by heat as soybean or cottonseed protein. For instance, the solubility of peanut protein in 1 *M* sodium chloride is not significantly decreased by dry heat at 118° for 2½ hours (282), in contrast to a 50% loss in solubility with soybean protein under approximately the same conditions (see curve 10, Fig. 4¹). Moist heat, however, at temperatures exceeding 80°, causes rapid denaturation of peanut protein (282). The solubility of the protein in 1 *M* sodium chloride of a series of commercial hydraulic-pressed meals ranged from 35 to 86%. The latter figure was comparable to values obtained with solvent-extracted meals prepared in the laboratory under mild conditions. A number of commercial meals also gave peptization curves (solubility of the protein in water as a function of *pH*) which were similar to solvent-extracted laboratory samples (283). Other commercial meals, of course, displayed lessened peptization over a wide *pH* range. It would appear that the commercial production of peanut meals with high protein solubility is within the realm of possibility under carefully controlled conditions.

d. Pea Meal

Evans and St. John (163) found that autoclaving pea meal at 110° for 30 minutes increased the glutelin fraction of the protein (soluble in 0.2% potassium hydroxide) and, at the same time, decreased the amount of protein which could be extracted with water or various salt solutions.

e. Wheat Gluten

The minimum solubility of wheat gluten in 95% ethyl alcohol and 0.1 *N* acetic acid (*v/v* 1:5) occurred when the protein was dry-heated at 153° for 18 hours (275). Increased solubility at higher temperatures was considered to be the result of the formation of soluble degradation products.

3. Destruction of Enzymes and Antinutritional Factors

The thermal denaturation of minor protein constituents which are characterized by a specific type of biological activity has been the object of numerous studies attempting to correlate changes in such activity with concomitant changes in the nutritive value of vegetable protein meals. Although such parallelism does not in itself prove a cause and effect relationship, these activity measurements frequently provide a rapid, simple technique for evaluating the nutritive value of processed meals in lieu of costly, time-consuming biological studies. (See also Chapters 7 and 14.)

a. Urease

Caskey and Knapp (284) reported that the heat treatment required for producing the maximum nutritional value of soybean oil meal was

the same as that required to inactivate the enzyme urease. A more extensive collaborative study (285) confirmed the fact that meals of low nutritive value because of inadequate heating generally gave a positive urease test. Borchers *et al.* (286), however, observed that urease was more sensitive to heat inactivation than the trypsin inhibitor (see below) and should therefore be regarded as unsatisfactory for testing the adequacy of heat treatment for soybean meals. It is likewise evident that the urease test is of little value in detecting overheated meals (286, 287).

b. Trypsin Inhibitor

Borchers *et al.* (286) have reported that the trypsin inhibitory activity of a solvent-extracted meal could be destroyed by exposure to flowing steam for 60 minutes or by autoclaving under the following conditions: 5 pounds for 45 minutes, 10 pounds for 30 minutes, 15 pounds for 20 minutes, or 20 pounds for 10 minutes. Borchers *et al.* (288) later found that the trypsin inhibitor was associated with meals which had been inadequately heated to achieve optimal nutritive value. Westfall and Hauge (19) showed that the nutritive value of partially heated soybean flours was inversely proportional to their trypsin inhibitor content. Of more fundamental interest are the studies of the kinetics and thermodynamics of the heat inactivation of crystalline soybean trypsin inhibitor (59).

c. Hemagglutinin

The destruction of the hemagglutinin in soybean oil meal has been found to parallel the improvement in the nutritive value effected by heat (289). Hemagglutinating activity has therefore been proposed as an index of the nutritive value of soybean oil meals. An improved procedure for measuring the hemagglutinating activity of soybean meals has been recently developed (290).

4. Electrophoretic Behavior

a. Soybean Protein

Mann and Briggs (291) studied the effect of heat on the electrophoretic behavior of the proteins in aqueous extracts of defatted soybean meal. The electrophoretic characteristics of the globulin fraction were not significantly altered by heating such extracts at 75° for 5 hours. The non-globulin fraction, however, migrated as a single peak after heating, compared to the heterogeneous system obtained with the unheated extract (Fig. 5). This change was attributed to an interaction of the minor components of the non-globulin fraction under the in-

fluence of heat to form an electrophoretically homogeneous complex. Since the same phenomenon was observed to occur when aqueous extracts were allowed to stand at room temperature for 5 to 7 days, it was believed that the effect of heat was to accelerate a kind of denaturation reaction which is capable of proceeding at lower temperatures.

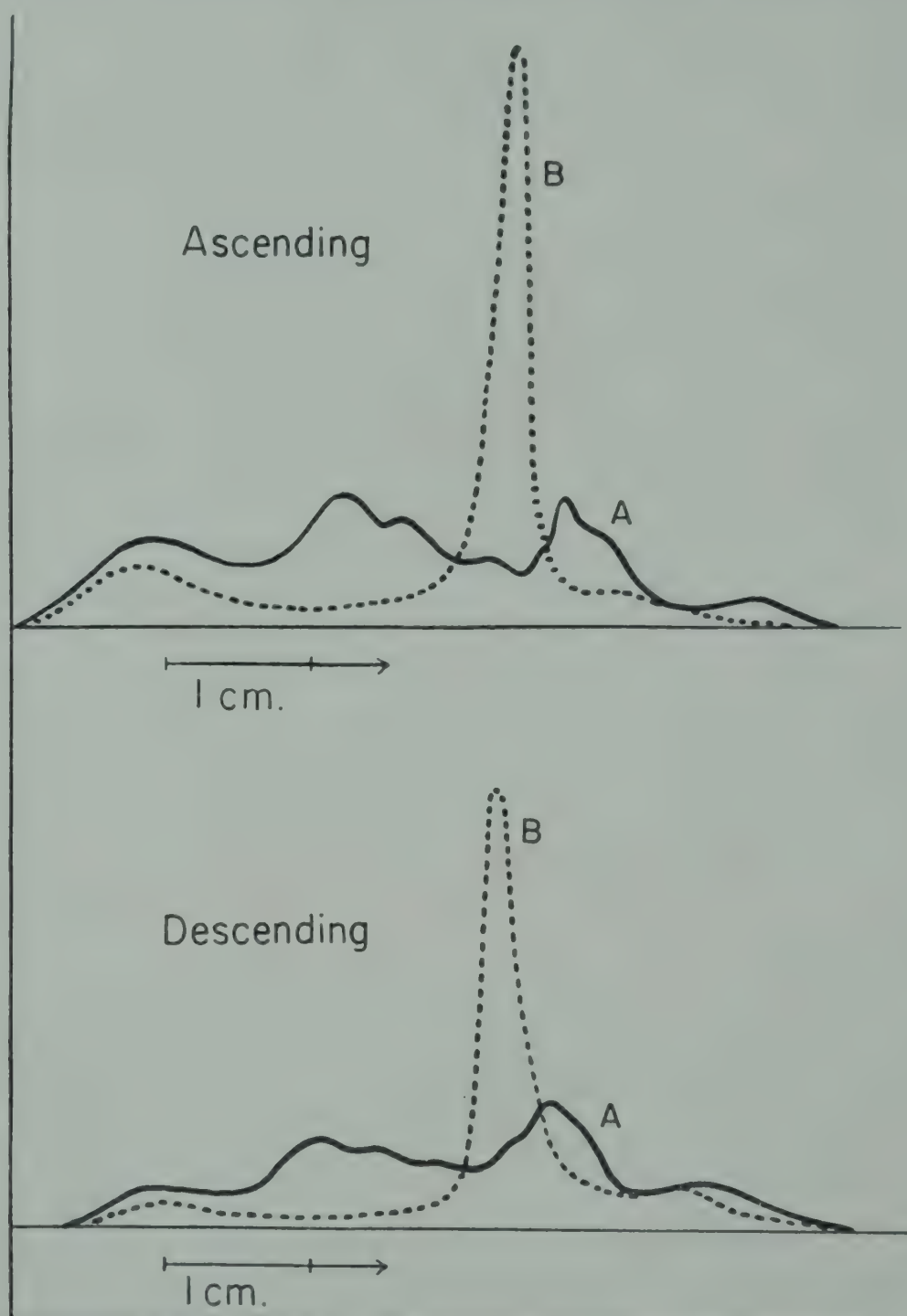


FIG. 5. Electrophoretic pattern of the non-globulin fraction of soybean extract before heating (A) and after heating (B) for 5 hours at 75° (291). Upper curve is ascending boundary, and lower curve is descending boundary. (Reproduced through the courtesy of *Cereal Chem.*)

b. Cottonseed Protein

The electrophoretic properties of the proteins extracted from cottonseed meals with ethylamine-barbital buffer (pH 10.4) were studied by Karon *et al.* (292). Two major components were observed with proteins extracted from a solvent-extracted meal subjected to a minimum amount of heat treatment. Screw-pressed meals which had been ex-

posed to more severe heat treatment during processing yielded protein solutions with electrophoretic patterns in which these two components were more difficult to resolve and in which there was a new fast-moving component. These effects are well illustrated in Fig. 6, taken from a paper by Condon *et al.* (187) dealing with the effects of autoclaving on the properties of solvent-extracted cottonseed meal. The electrophoretic patterns of those meals which had undergone no reduction in nutritive value (autoclaved up to 15 minutes) were similar to the pattern of the unautoclaved meal showing two distinct components, A and B.

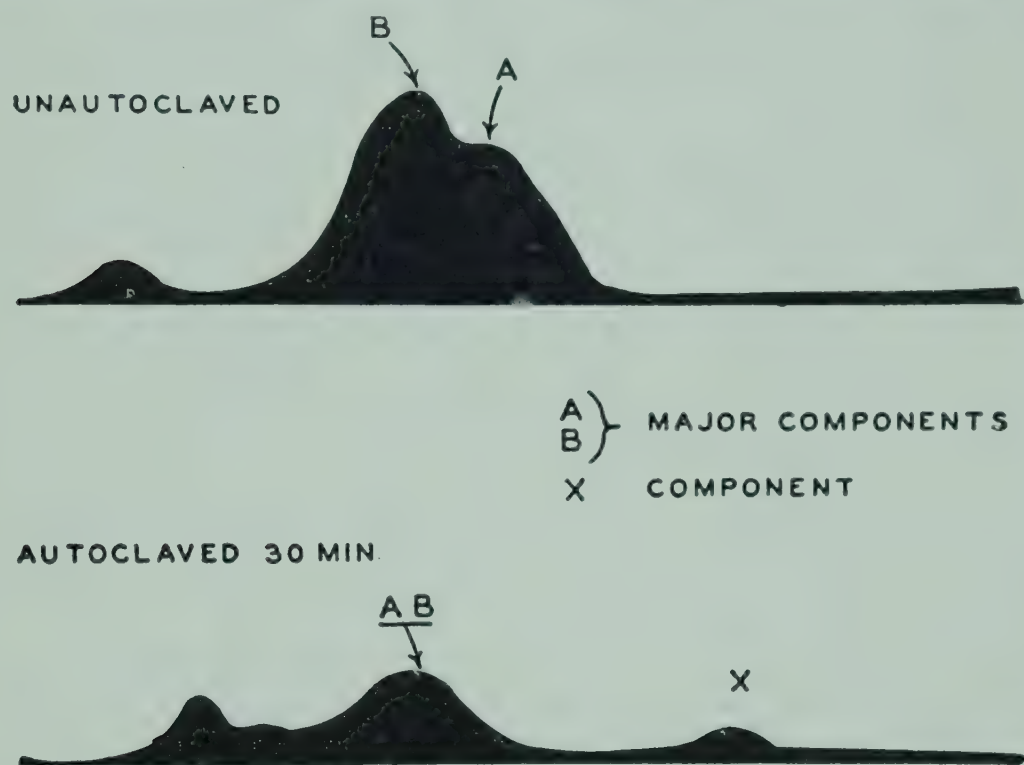


FIG. 6. Representative electrophoretic patterns of cottonseed meals of high and low protein quality index (187). (Reproduced through the courtesy of *J. Agr. Food Chem.*)

More prolonged heating which caused a decrease in nutritive value caused these two components to merge with the concomitant formation of a new component, X.

V. IMPORTANCE OF HEAT TREATMENT IN THE INDUSTRIAL USAGE OF VEGETABLE PROTEINS

1. Soybean Protein

In addition to its use in livestock feeding, soybean protein in the form of flour, flakes, or grits, or in an isolated form, has been used in the manufacture of a wide variety of food and industrial products (293). (See also Chapters 10, 11 and 15.)

a. Food Products

(1) *Baking industry.* The use of soybean flour in bread formulation has received considerable attention because soybean proteins provide an excellent means of supplementing the inherent lysine deficiency of wheat protein. Since

soybean flour prepared by the screw-press process has poor baking characteristics (294), full-fat or defatted soybean flour prepared by the solvent-extraction process has been used almost exclusively for bread making. These flours may be added to bread formula up to a level of 3% without any noticeable effect on the baking properties of the dough. In excess of this level, however, soybean flour may cause a deterioration in bread volume, crumb character, and dough handling properties—difficulties which can be largely circumvented by the proper use of oxidizing agents (294–297). Ofelt and his associates have shown that most commercial soybean flours have received sufficient heat treatment to inactivate the proteases (298) and amylases (299), indicating that these enzymes are probably not responsible for the undesirable effects produced in the absence of oxidizing agents. Further research is obviously needed to establish the factors responsible for the changes in baking characteristics produced by soybean flour.

Soybean flour or soybean extracts may be used as a bleaching agent for wheat flour. This bleaching action is due to the presence of the enzyme lipoxidase, which has the ability to destroy the colored carotenoid pigments of unbleached flour (300, 301). Lipoxidase is very sensitive to heat inactivation so that the application of heat must be carefully controlled during the processing of soybean flour intended for use as a bleaching agent. Sumner and Tressler (302) found that screw-pressed and solvent-extracted meals processed at maximum temperatures of 150° and 100°, respectively, were devoid of lipoxidase activity, whereas raw soybeans and a solvent-extracted meal processed at 65° had lipoxidase activity of 12,650 and 6800 units respectively.

(2) *Meat industry.* Soybean flour is widely used in the meat industry as a binder and emulsifier in the preparation of sausages, meat loaves, and related products. Burnett (293) has described the experience of Tauber relating to the use of various types of soybean flours in the manufacture of sausages. Low-fat flour prepared by the screw-press process gave sausages which had a definite burnt flavor presumably due to the excessive heat treatment received during processing of the flour. Solvent-extracted meals which had not received any heat during processing imparted to the sausage a “beany” flavor and a slight solvent taste. Sausages prepared from a soybean flour which had been treated with only sufficient heat and moisture to remove the “beany” flavor were acceptable with respect to odor, appearance, and taste.

(3) *Brewing industry.* The use of soybean protein in the form of flour, flakes, or grits has been advocated for use in the brewing industry to improve the body and flavor of beer, to increase foam stability, and to stimulate yeast growth. Hayward (303) and Wahl (304) have emphasized the desirability of using soybean preparations which have been subjected to a minimum amount of heat treatment in order not to depress the solubility and foam-producing properties of the protein. The protein of the soybean flakes recommended by Hayward (303) had a solubility of 80% in water, this high value being indicative of the mild processing conditions used in the preparation of this product.

(4) *Sugar industry.* Chang (305) has described a novel use for soybean flour for the defecation of the sugar liquor in the refining of sugar. Since the water-soluble protein is believed to be responsible for the defecation function, it follows that soybean flour receiving as little heat as possible is to be preferred.

(5) *Other uses.* Isolated and modified soybean protein fractions have been used in the manufacture of aerating agents for confections and toppings, as ingredients in candy and ice cream, and in the preparation of a number of Oriental foods. The preparation of the protein fractions used in such products in satisfactory yield and quality requires as starting materials soybean flours which have received as little heat treatment as possible so as not to interfere with the solubility of the protein.

b. Industrial Uses

(1) *Isolated protein.* Isolated soybean protein is used for a wide variety of purposes which include wallpaper coating, water and latex paints, lamination of fiber board, fibers, fire-foam liquids, printing inks, leather finishing, felt-base floor covering, sizing, and adhesives (2). Meals which have been processed by screw- and hydraulic-press equipment are excluded as starting materials for protein isolation owing to the denaturation and discoloration of the protein caused by the elevated temperatures employed in such processes. The raw material for the preparation of soybean protein is thus limited almost exclusively to solvent-extracted meals.

(2) *Soybean oil meal.* For some industrial purposes the meal rather than the isolated protein may be used. Soybean meal used for making wallpaper adhesives (306) and plywood glues (293) must have a high degree of protein solubility. In some instances, however, soybean meals with insoluble protein are to be preferred. Soybean meal with undenatured protein is unsatisfactory for use in plastics because of its lack of water resistance (307). To overcome this difficulty, soybean meal was leached at its isoelectric point and heated under pressure at 105° to 120° for 2 to 3 hours. This leached, denatured meal could be used to replace up to 20% of the wood flour in phenolic plastics, and was also found to be satisfactory as an extender in phenolic plywood glues (308).

2. Cottonseed Protein

A considerable amount of research has been conducted in this country on the properties of cottonseed protein for the purpose of ascertaining its suitability for industrial application (309). Solubility considerations dictate that only solvent-extracted cottonseed meal would be the most practical raw material for the isolation of the protein. Even in those industrial applications where cottonseed meal could be used instead of the isolated protein, as in the manufacture of plywood glues, the solvent-extracted meal was found to be superior to hydraulic- and screw-pressed meals (310).

3. Peanut Protein

Isolated peanut protein may be used for many of the same purposes as soybean protein (2, 311). A study of the solubility properties of commercial hydraulic-pressed peanut meals has demonstrated that such meals when prepared under suitable conditions may be quite suitable for isolation of the protein (283). Solvent-extracted meals are to be preferred, however, because they can be used directly for isolation purposes, whereas meals prepared by hydraulic pressure must first be ground and the fines removed by sifting

(311). Excessively heated meals require a higher alkalinity to disperse the protein than meals prepared under milder conditions. Proteins prepared in this manner may be undesirable for certain uses, as in the manufacture of paper coatings where a high pH was found to weaken the raw paper stock (312).

The fact that the protein of some hydraulic-pressed meals may be as soluble as the protein from solvent-extracted meals does not exclude the possibility that differences in other properties may exist. Burnett *et al.* (313) found that the protein extracted from hydraulic-pressed meals at the same pH and to the same extent as the protein from an uncooked solvent-extracted meal had a higher viscosity than the latter. Since proteins which are to be used as adhesives must possess a stable and low viscosity (314), it is evident that the protein extracted from hydraulic-pressed meals is less desirable for this purpose than that from solvent-extracted meals.

VI. CONCLUSIONS

There are limitations in the extent to which one is justified in drawing broad generalizations on a topic as diversified as that considered here. Nevertheless, there appears to be a sufficient amount of related information to permit one to draw an integrated picture of this whole problem.

The beneficial effect which proper heat treatment exerts on the nutritive value of some vegetable protein meals is related to the concomitant inactivation of specific heat-labile factors which elicit deleterious physiological responses in animals. For convenience these have been summarized in Table IX. In some cases (for example, the trypsin inhibitor and hemagglutinin of soybeans, free gossypol of the cottonseed, and the goitrogenic factor of rapeseed) these factors have been well characterized, but in many instances little is known about them except that they do exist.

The impairment in nutritive value which follows the excessive application of heat is associated with certain profound changes in the protein molecule itself or changes resulting from the interaction of the protein with carbohydrate-like components which accompany the protein in crude meals. In either event these modified proteins become more refractory to enzymatic (trypsin) attack with a consequent retardation in the rate with which the essential amino acids are released during digestion. The formation of peptide linkages which cannot be split by trypsin may arise in two ways: (1) the interaction of the non-peptide carboxyl groups of glutamic and aspartic acids with the non-peptide amino groups of lysine or arginine giving rise to atypical peptide linkages, and (2) the interaction of the non-peptide amino group of lysine and arginine with reducing sugars so modifying the substrate that it no longer conforms to the specificity requirements of trypsin. More severe heat treatment causes reaction 2 to proceed to the

point where the basic amino acids can no longer be completely recovered even by acid hydrolysis (i.e., destruction).

In the animal organism the decreased protein digestibility induced by heat may lead to the excretion of substantial portions of the protein, thus depriving the animal of the amino acids contained therein. If one of these amino acids is limiting in the original protein, the animal will

TABLE IX
HEAT-LABILE ANTINUTRITIONAL FACTORS IN VEGETABLE PROTEIN MEALS

Vegetable protein meal	Antinutritional factor
Soybean oil meal	Trypsin inhibitor Hemagglutinin Saponin Goitrogenic factor Anticoagulant factor Diuretic principle Lipoxidase
Cottonseed meal	Gossypol ^a
Other legumes	Trypsin inhibitor Hemagglutinin Toxic factor (kidney beans)
Linseed meal	Antipyridoxine factor Cyanogenetic glycoside
Rapeseed meal	Goitrogenic factor
Mustard seed meal	Toxic glycoside
Tung meal	Saponin and/or protein ^b
Castor meal	Ricin

^a Gossypol is not heat-labile, but its detoxification to form "bound" gossypol is promoted by heat.

^b See discussion in Chapter 31.

suffer from a deficiency of this particular nutrient. Thus, soybean oil meal, which is suboptimal with respect to methionine, becomes critically deficient in this amino acid when overheated, and, similarly, excessive heat treatment causes the marginal levels of lysine in cereal products and cottonseed meal to become critical deficiencies. The destruction of lysine further accentuates the critical need for this amino acid and, in certain instances, may even precipitate a lysine deficiency with proteins which are normally considered fairly good sources of lysine.

The denaturation of protein by heat is characterized by lessened solubility in aqueous solvents. This effect is of particular importance in the industrial utilization of vegetable protein meals where the protein must be highly dispersible if it is to be isolated in satisfactory yield and quality. Vegetable protein meals intended for industrial usage are in most instances limited to solvent-extracted meals which have received a minimum amount of heat treatment.

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CHAPTER 6

EFFECT OF OTHER PROCESSING FACTORS ON VEGETABLE PROTEIN MEALS

K. A. KUIKEN

I. INTRODUCTION

This chapter is concerned with the influence of processing factors, other than heat, on the nutritive value of vegetable protein meals. In selecting topics for discussion, "effect on nutritive value" was considered of primary importance, and definition of "processing factor" was treated liberally. Hence, there is included material which is not related directly to industrial processing but is of importance in feed utilization of vegetable protein meals.

The first subject is the general field of solvent extraction, where high-quality protein products are available, but feed utilization is sometimes restricted by physical properties and fiber content of meals. Choice of solvent also is shown to modify nutritive value of selected products.

Three examples of chemical contamination of feed products are cited. This discussion is followed by reference to agronomic research on the selection of varieties and strains of seeds for improved nutritive quality. Consideration is then given to the possible effects on nutritive quality of meals resulting from storage of raw materials and finished products.

II. SOLVENT EXTRACTION OF OILSEEDS

1. Introduction

The oilseed processing industry is developing in the direction of extensive application of solvent-extraction methods as a result of economic factors which penalize the older mechanical methods of oil recovery. Conversion of the soybean industry in the United States to solvent processing is far advanced; the volume of solvent-extracted cottonseed and linseed oil meals is increasing rapidly; other solvent-extracted products, such as copra and peanut meals, are available.

The purpose of the following discussion is to illustrate how solvent processing and selection of specific solvents can modify use of oilseed meals in feeds and affect nutritive value of these products. In general, physical properties of meals are altered but protein quality is usually improved; in specific instances, behavior of toxic entities and selective solvent action on meal components is of importance.

2. Physical Properties of Solvent-Extracted Oilseed Meals

Seed meats which are relatively high in oil content, such as cottonseed, peanuts, linseed, and copra, have a friable structure. For these, solvent extraction removes residual lipid to the point where dustiness and lightness of meal particles become a problem. The entire industry, from the original seed processor to the final consumer, has had to reconsider such factors as tightness of bags, bins and mixing equipment, methods of transport, and segregation of blended products. Pelleting operations often require development of special techniques to attain satisfactory production rates and acceptable pellet quality.

There are two general approaches to this physical problem that are in use. The first is to prepare a product of controlled particle size with a rather coarse texture. The products might be termed grits, screenings, or crumbles. They are made by breaking pellets, or other compacted material, and then screening to size. The alternate practice is to add selected oil fractions back to extracted meals. This can be easily accomplished during the normal desolventizing operation if refinery fractions are locally available. Soapstock is the fraction of particular interest, since the quantities available at low cost have increased because of the change in balance of the detergent-soap industry. Curtin and Raper (1) refer to addition of acidulated cottonseed soapstock (hydrolyzed cottonseed fat) to solvent-extracted cottonseed oil meal. Whittecar (2) describes addition to meal of cottonseed phosphatides obtained by water washing of oil. Continued development in this direction is expected to resolve completely the dust problem.

Each of the approaches to dust control appears to improve nutritive quality of meals by increasing feed consumption. Addition of fat also has the advantage of increasing the net energy content of the feed. Both processes tend to result in darkening of the color of vegetable meals. These color changes usually have no relationship to nutritive value of the product, but they do represent a change in arbitrary standards which consumer and trading organizations have adopted through experience. Educational effort is required to interpret correctly these color problems.

B. Protein Quality and Content

As a general rule, the highest quality vegetable protein meals are obtainable under conditions of minimum heat treatment. Processing without heat, however, may not be ideal, because some complex vegetable protein systems contain heat-labile substances which interfere with protein utilization. For example, most beans contain enzymes which must be heat-inactivated for optimum development of protein nutritive value. Since solvent-extraction methods avoid cooking procedures typical of hydraulic- and screw-press methods, heat damage to protein is minimized in solvent operations; controlled mild heating then can be applied where necessary to inactivate undesirable components. The net result is that solvent processes are expected to provide a product of high protein quality for "critical animal" feeds.

The term "critical animal" refers to the monogastric animals which have definite requirements for individual amino acids and are unable to utilize simple nitrogen compounds for effective protein synthesis. In contrast with this group of animals are the ruminates, which are far less discriminate in their requirement for dietary amino acids. This gross difference in dietary requirement is a result of bacterial action in the rumen; the bacteria effectively degrade crude proteins and synthesize amino acids for their host from a variety of sources. For the same reason, ruminates are able to utilize high-roughage feeds, whereas the monogastric animals require low-roughage rations. Hence, if improved protein quality products from solvent mills are to be used in "critical animal" feeds, these oilseed meals should have a low crude fiber content.

Raw materials which contain a large hull fraction must be cleaned thoroughly to provide products of high protein content with low fiber. The situation is most critical in the cottonseed industry, because cottonseed oil meal is the volume product which can make up the deficit of high-quality vegetable proteins in the southern part of the United States. High-quality protein meals are now available from this source, but the economics of the industry have developed around products containing 36 and 41% protein, and about 15 and 12% crude fiber from hulls. Certainly a product containing 44 to 50% protein is reasonable. New uses for hulls from all major vegetable seed sources must be developed, however, to supplement the knowledge available on improved protein products and to stimulate efforts to produce meals of higher protein content. It is necessary also to improve the efficiency of the mechanical methods for separating seed meats from hulls. The primary control problem is to process under conditions which produce intact meats and large clean hull particles. Hull scalping by screening and pneumatic cleaning invariably results in costly protein and oil loss to

the hull fraction when applied to seed fragments and finely divided material.

Equipment for hull removal is specialized, and for this reason the experimental or practical operation of a solvent-extraction oilseed mill with a variety of raw materials may not be satisfactory; however, in areas of adequate seed supply, one usually finds mills equipped to process alternately at least two seed crops.

4. Solvents for Oilseed Extraction

a. Hydrocarbons

Low-boiling petroleum fractions are the solvents most commonly used by the vegetable oil extraction industry in the United States. Commercial hexane is the fraction usually employed. The extensive literature which has developed on application of this solvent is supplemented by reports on oil-extraction characteristics of a variety of pure hydrocarbons in the C-5 to C-7 range (3, 4), hexane-alcohol azeotropes (5), and a hexane-water cosolvent system (6). These special hydrocarbon systems do not offer advantages to encourage significant industrial interest. Development of a satisfactory solvent process based on a less flammable solvent would merit great attention.

Cottonseed pigment behavior. No nutritional problems are known to be attributable to use of hexane *per se* for oilseed extraction. A problem does occur in the cottonseed industry, however, because dry hydrocarbons do not remove the natural toxic material from these seeds. This is of no significance in feeding ruminant animals, but it is of importance in feeding swine and poultry. Various solutions to the problem are available, and superior-quality cottonseed meal is being produced commercially by hexane extraction. The gossypol problem is discussed in Chapter 17.

Boatner and co-workers have explained why hydrocarbons do not remove the natural toxic compounds of cottonseed by demonstrating that these components occur entirely within pigment glands having a gelatinous carbohydrate wall (7, 8) which is resistant to hexane and many other solvents (9). If the glands are not ruptured by chemical or physical processing methods, the intact structures carry their toxic contents into the finished meal.

The prepress method of cottonseed extraction employs a screw press for preliminary removal of oil and then hexane extraction of the press cake to complete the process. Pigment gland rupture is accomplished by shearing action in the screw press (10). After the glands are broken, the hydrocarbon solvents may readily extract gossypol, a toxic pigment which accounts for about 50% of the weight of the pigment glands (11). The degree of solvent extraction of gossypol in this process is modified by heat and moisture control,

however, because moist heating effectively ruptures pigment glands and renders gossypol non-extractable by chemically binding the pigment to meal components (12). An excellent review of these phenomena has been published by Boatner *et al.* (13).

A survey of processing conditions and meal characteristics for eleven pre-press-solvent cottonseed mills has been reported by Pons *et al.* (14). Representative samples of meal obtained in the Pons survey were evaluated in nutrition studies by independent methods in four laboratories. Joint publication of the data on nutritive value (15) indicates that a wide variety of results can be obtained by this processing method. All samples were sufficiently low in free gossypol content to be considered non-toxic for practical feeding of growing swine and poultry. Protein quality was directly related to the cooking procedures employed prior to pressing. In cases involving mild cooking, the protein quality closely approximated ideal laboratory standards.*

Direct extraction of cottonseed with hexane does not require cooking and, therefore, affords excellent opportunity to obtain high-quality protein meal if secondary treatments are employed to remove or inactivate gossypol. Several treatments which can be applied in the normal solvent-removal operation have been described in the patent literature. Bonotto (16) used ammonium carbonate to supply ammonia vapor for gossypol inactivation and reported residual free gossypol values of 0.05% in treated cottonseed meal. In a later patent Bonotto (17) described a process employing sulfur dioxide in steam. Rice (18) used methanol containing 5% water to rupture pigment glands after hexane extraction. This was patented as an improvement over a previous process by Hutchins and Williamson (19) which employed a mixture of hexane and methanol for the same purpose. Rice (20) has also described a process which employs an organic amine to form insoluble gossypol addition compounds after gland rupture with water.

Amine-treated cottonseed meal is the only low gossypol meal of the direct-extracted type known to be in commercial production and to be characterized in reported animal nutrition studies. High protein quality has been demonstrated in broiler (21, 22) and turkey poult growth studies. Toxicity has not been observed with this product.† Experience with parakeratosis, however, has delayed progress in swine feeding studies (24).

b. Alcohols

(1) *Ethanol*. Japanese workers have reported use of ethanol for extraction of soybeans in a mill at Darien, Manchuria (25). Industrial development of alcohol-extraction systems has not occurred in the United States. The early Japanese interest in alcohol extraction was a result of local availability of solvents; at the present time, Japanese processors generally use hexane for extraction of soybean oil (26).

* The standard reference meal was prepared by hexane extraction of oil followed by butanone extraction of pigments at room temperature.

† The official methods of the American Oil Chemists' Society for gossypol analysis are not directly applicable to amine-treated cottonseed meal. See Pons and Hoffpauir (23).

The lower aliphatic alcohols are relatively poor solvents for glyceride oils at room temperature. At elevated temperatures the alcohols and oils form miscible systems which separate on cooling. Beckel and co-workers (27–31) have reported extensive developmental work on an ethanol-extraction system for soybeans and have arrived at a non-distillation process based on these temperature-solubility characteristics.

Ethanol effectively removes pigments and simple sugars which tend to darken commercial soybean meals. The light color of alcohol-extracted meal is a definite advantage if the meal is to be used for isolation of industrial soybean protein or for manufacture of edible products. A further advantage for edible end uses is that ethanol removes the bitter and beany flavor components of soybeans (28, 32). Hence, alcoholic extraction of defatted soybean flakes is used in the production of Gelsoy, an edible soybean protein (33, 34). Protein meals of excellent nutritional quality are obtained by alcohol extraction.

Recent data on alcohol extraction of oil from cottonseed, peanuts, sesame, and soybeans have been reported by Rao *et al.* (35).

(2) *Isopropyl alcohol*. Harris has reported development work on the extraction of cottonseed with isopropyl alcohol (36–38). Phase equilibrium data for cottonseed oil in ethanol and isopropyl alcohol also have been reported by Magne and Skau (39).

The Harris method for isopropyl alcohol extraction of cottonseed produces meals containing very low levels of free gossypol. A representative meal having a free gossypol content of 0.003% was fed to hens at a 20% dietary level. No visual discoloration was observed in eggs stored for six months (40).

c. Trichloroethylene

(1) *History*. Trichloroethylene has been used for commercial extraction of soybeans in various parts of the world. The experience can be divided into three historical periods. In each instance toxicity of trichloroethylene-extracted soybean oil meal (trichlor meal) was noted in cattle and processing was discontinued.

Stockman (41) was the first to describe trichlor meal toxicity. He attributed cattle deaths in Scotland in 1916 to feeding of soybean oil meal processed by trichloroethylene extraction. Later experience in Germany was described in 1927 by Stang (42), who used the term "Duren disease" when referring to the problem. The term "Brabant disease" has also been used in related European literature (43).

The early European literature on toxicity of trichlor meal is much more extensive than is indicated here. Nevertheless, the information

was not well known when renewed interest in trichloroethylene extraction of soybeans developed in Italy, the United States, and Japan around 1950. This interest was stimulated by commercial availability of an apparently satisfactory solvent which would permit great economy in mill design because the flammability problem could be avoided. Toxicity to cattle occurred in these modern cases, and the reports which appeared (43-48) were essentially identical with the early descriptions of Stockman (41), Stang (42), and others. The net result was that maximum production of trichlor meal reached an estimated 2% of total production in 1952 (49), only one small domestic mill was reported to be operating a "trichlor" process in 1955 (50).

(2) *Biological effects of feeding trichloroethylene-extracted meal.* The toxicity symptoms of trichlor meal observed in cattle are an extreme reduction in white blood cell count, a high temperature, eventual bleeding at the natural body openings, and severe general internal hemorrhage. Stockman (51) pointed out that identical symptoms occur in cattle poisoned by eating bracken ferns. To date, it is impossible to distinguish between the two conditions without knowledge of dietary history.

The condition can be produced experimentally in cattle by feeding about 2 pounds of trichlor meal per day for a period of one to six months (47). Toxicity has been observed in sheep, but the characteristic white blood cell change was not noted (52). Attempts to produce toxic symptoms in convenient small laboratory animals have not been successful. Balloun (53) did not find toxic effects when trichlor meal was fed to chicks; Becker and associates (54) did not observe toxicity in swine.

Stockman, and also Stang, was unable to demonstrate the toxic symptoms in cattle by feeding trichloroethylene with soybean meal processed by other methods. Modern investigators have confirmed these observations. McKinney and associates (55) suggested the possibility of reaction between meal components and autoxidation products of trichloroethylene as a possible explanation for development of toxicity. They accordingly studied this chemistry and identified the normal oxidation products. In a subsequent paper (56), feeding experiments were reported for soybean flakes which had been treated with oxidation products of trichloroethylene. Although the results were inconclusive, there is reason to continue this work. Of particular interest was observation of leucopenia in a calf fed casein which had been treated with oxidized trichloroethylene. "The results with the casein preparation suggest that the soybean *per se* may not be essential to the formation of the toxic entity and that under suitable processing conditions other

oilseeds, meat products, and proteinaceous materials might produce toxic products." (See also Chapter 14.)

(3) *Literature on processing with chlorinated solvents.* Engineering aspects of oilseed extraction with trichloroethylene have been studied by Arnold and associates (57–65). Their work includes evaluation of other chlorinated solvents, i.e., methyl chloroform, 1,2,3-trichloropropane, ethylene dichloride, and dichloromethane (64). The possible production of cottonseed meal with low free gossypol content and a minimum of heat-denatured protein by trichloroethylene extraction has been indicated (65). Nutrition studies have not been reported for oilseed meals prepared with the solvents listed above or for trichlor meals other than those prepared from soybeans.

III. CHEMICAL CONTAMINATION OF FEED PRODUCTS

Accidental chemical contamination can have serious effects on the nutritive value of feed products. The purpose of this discussion is to illustrate the problem by citing three cases that have been reported in the literature. In two of these cases, the pelleting operation proved to be a source of unexpected chemical contamination. The third case illustrates the importance of adequate labeling and education in the practical use of chemically treated products. Great advances have been made in crop and animal production through expanded use of agricultural chemicals, and the combined industry has done a commendable job of controlling possible hazards of this type.

1. Bovine Hyperkeratosis (X-Disease)

a. Occurrence

X-disease, or hyperkeratosis of cattle, was first recognized and described by Olafson (66) of Cornell University in 1947. The condition developed rapidly throughout the United States (67) and became the most costly feed contamination problem on record. Sikes *et al.* (68) estimated cattle losses attributable to hyperkeratosis in Tennessee to amount to more than \$1,000,000 by 1952. More than 3000 cattle were involved in a single instance of feed contamination in Texas reported in 1953 (69).

b. Causative Agent

The causative agent involved in X-disease has been identified through the work of Sikes *et al.* (68, 70) and Bell (71) as highly chlorinated naphthalene. Special gear lubricants for high-pressure applica-

ion have been the source of the toxicant in most cases. The feed industry experienced the problem because of application of these lubricants in pellet mills prior to development of the information on toxicity. The toxic naphthalenes may also be found in wood preservatives and electrical insulation. Wood preservatives have been the source of the toxicant in European experience.

Copenhaver and Bell (72) have demonstrated conclusively that lubrication of a pellet mill with a grease containing 3% chlorinated naphthalene will result in toxic feed. A practical occurrence of the condition attributable to pelleted dehydrated alfalfa was reported by Olson *et al.* (73). The Texas experience (69) involved manufacture of cottonseed meal pellets under conditions similar to those described by Copenhaver. Isolation of pentachloronaphthalene from cottonseed pellets has been reported (74). Isolated occurrence of hyperkeratosis (68) can occur through carelessness with lubricants on the farm.

Wagener (75) produced hyperkeratosis in Germany by exposing cattle to a complex wood preservative used in construction of a new barn. Sikes later stored a feed concentrate in a room which had been painted with chlorinated naphthalenes and demonstrated toxicity in cattle as a result of feeding the concentrate after exposure to the vapors in the room (76). At the present time it is believed that wood preservatives containing chlorinated naphthalenes are not being produced in the United States. Nevertheless, one should exercise caution in application of wood preservatives in feed bins and animal shelters.

Application of lubricants containing chlorinated naphthalenes to the skin will produce bovine hyperkeratosis (68). The toxic agent is passed from dam to calf through the milk (68).

c. Toxic Level

The toxic level of the chlorinated naphthalenes is very low, and the effects of repeated administration are accumulative. Bell (77) studied naphthalene isomers containing from two to eight chlorine atoms. The isomers containing two and three chlorine atoms were not toxic at a total dose of 12 mg. per pound of body weight, administered over a 2-day period. Slight toxic symptoms were noted for the Cl-4 isomers at an equivalent dose level. The Cl-5, Cl-6, and Cl-7 isomers were consistently toxic at a total dose of 2.5 mg. per pound of body weight.

The experiments of Sikes *et al.* (68), which were not intended to establish minimum toxic levels, included the following dose-response observations: (1) A 450-pound Jersey bull calf fed 1 g. of octachloronaphthalene per day for 13 days died on the fifty-seventh day; (2) a

200-pound Guernsey calf which received 5 g. of a chassis lubricant per day for 16 days died on the thirtieth day.

d. Toxicity Symptoms

The term hyperkeratosis is descriptive of skin changes seen in affected animals. Skin on the side of the neck, across the withers, and around the mammary gland may become dry, hard, stiff, and drawn up into rolls which later develop fissures. This skin change usually occurs in chronic cases but may be completely absent in acute cases. Wart-like proliferations may develop on the lips, tongue, and hard palate.

The gross symptoms always observed are excessive lacrimation, diarrhea, polyuria, marked salivation, and a serous discharge from the nostrils. Spontaneous abortion occurs in pregnant animals. Necropsy indicates specific lesions in the mouth, intestinal tract, and bile duct. Degeneration of the liver, pancreas, kidneys, and other tissues has been described (68).

The toxicity symptoms are almost identical with the effects of vitamin A deficiency. In fact, blood plasma vitamin A levels are always below normal in affected animals (78, 79). The decrease in plasma vitamin A apparently precedes other symptoms of hyperkeratosis. Administration of large amounts of vitamin A, however, does not prevent development of toxicity symptoms or fatal termination in cases of chlorinated naphthalene poisoning.

2. Pellets Containing Stilbesterol

Stilbesterol is of interest as a cattle feed ingredient; it is reported to stimulate rate of weight gain and reduce feed cost (80). On the other hand, low-level contamination of feeds with stilbesterol can cause serious reproductive disturbances in some animals. Hadlow *et al.* (81) have reported scrotal hernia and persistent estrus in mice as a result of pelleting the feed in a mill which had been used to prepare cattle feed pellets containing stilbesterol. Their observations indicate that it is very difficult to remove all traces of the hormone from a pellet mill.

3. Chemical Seed Protectants and Pesticides

Two reports indicate an extreme toxicity of Arasan (tetramethyl thiuram disulfide) to chickens (82, 83). Arasan is a seed protectant intended for application only to planting seed. Nevertheless, one of these reports [Waibel *et al.* (83)] actually concerns experiences with contaminated corn involving 75,000 hens on six farms in Minnesota. Normal production in laying birds stopped. Soft-shelled and misshapen

eggs were found. (A similar egg condition occurs in Newcastle disease, infectious bronchitis, and pullet disease.) Egg production problems were noted with 25 p.p.m. of active ingredient in the ration. Chicks failed to grow and exhibited leg abnormalities.

Carter *et al.* (84) call attention to the possibility that application of insecticides to forage crops may result in contamination of meat and milk products.

IV. SELECTION OF VARIETIES AND STRAINS OF SEEDS

The total protein content of seed crops is influenced significantly by genetic, environmental, and agronomic factors. The subject has been reviewed for soybeans by Morse (85) and for cottonseed by Tharp (86). Although these gross quantitative effects are of interest to nutrition, a question of greater importance is to determine whether or not essential amino acid distribution can be influenced favorably by variety selection. Where toxicity occurs, selection to reduce or eliminate toxicity is of particular interest. The following discussion briefly presents examples of research in this area. The experiments are generally complicated by variables that cannot be controlled with complete satisfaction. Progress is slow because of the time required for development of the selected plants, and the observed intervarietal differences are usually rather small. From the commercial point of view, the practice of pooling large volumes of the major agricultural crops for distribution and processing has the effect of completely masking intervarietal differences.

1. Amino Acids in Soybean Varieties

Methionine (87) is the limiting amino acid in soybean meals. Attention has been focused, therefore, on the sulfur amino acid content of soybean varieties. Csonka and Jones (88) suggested the possibility of selection for improved cystine content on the basis of analysis of sodium chloride extracts of defatted beans of six varieties. The cystine content of the extracts, expressed as per cent of total nitrogen distributed in cystine, ranged from 0.37 to 0.63%. Hamilton and Nakamura (89) obtained similar data on alkaline extracts for eleven soybean varieties and reported cystine values ranging from 0.38 to 1.04% (same nitrogen basis). Johanson and Lugg (90) later studied cystine and methionine in two bean varieties and observed differences in cystine content that were interpreted in terms of variation in non-protein amino acid; the cystine and methionine content of the bean proteins were essentially identical. No evidence of non-protein methio-

nine was obtained. Krober (91) suggests the possibility of selection of bean varieties for methionine content.

Kuiken and Lyman (92) reported on the essential amino acid content of defatted raw and toasted flakes obtained from twenty varieties of soybeans. Widest variation in essential amino acid content was noted in the case of methionine where the highest value was 19% above the lowest value. There was no indication that selection from the varieties studied would permit development of a superior food protein strain.

Everson and associates (93) evaluated nutritive value of raw and heated meals prepared from five varieties of soybeans. No appreciable differences were noted between varieties.

2. Amino Acids in Cereal Grain Varieties

Several reports concerning genetic control of protein content, amino acid distribution, and vitamin levels in cereal grains have appeared. The data of Frey and associates (94, 95) indicate genetic control of protein and B complex vitamins in oats. The possibility of developing oat strains with higher content of riboflavin and niacin is considered favorable. Doty *et al.* (96) studied corn crosses and reported that the amounts of tryptophan, tyrosine, cystine, arginine, and histidine were related to genetic constitution. Frey (97) analyzed corn crosses for protein and various amino acids and concluded that the amino acid distribution in his high-protein corn samples was less favorable for nutrition than the distribution in low-protein samples. A large number of corn samples were analyzed by Flynn *et al.* (98), who grouped the samples according to protein content into a high group averaging 14.3% and a low group averaging 9.9% protein. The average amounts of nutrient in milligrams per gram of protein in high- and low-protein corn were, respectively: tryptophan, 6.9 and 8.9; lysine, 26.6 and 31.1; methionine, 18.1 and 20.3; cystine, 12.8 and 14.6; nicotinic acid, 0.169 and 0.258. Csonka (99) studied white and yellow corn and concluded that cystine, tryptophan, tyrosine, arginine, histidine, and lysine were equally distributed in his samples.

Excellent data on the composition of the United States corn crop based on large numbers of samples obtained during the 1946 and 1947 seasons were published by the National Research Council (100).

3. Gossypol Content of Cotton Varieties

Schwartz and Alsberg (101) were early investigators in this field; they analyzed seed samples representing the entire cotton-growing area of the United States. The data indicated variation in gossypol content of individual varieties grown in the same location for successive seasons

and also variation with location in a selected season. Environmental factors appeared to mask genetic factors. The following general correlation was observed: seed from the Southwest were low in oil and gossypol content; seed from the Southeast were intermediate in oil and gossypol content; and seed from the Pacific Coast were highest in oil and gossypol.

More recent work has demonstrated that both environmental and genetic factors are involved in regulating the gossypol content of cottonseed. Gallup (102) studied an individual seed variety during successive seasons at various locations in Oklahoma and noted that high rainfall appeared to favor an increase in oil and gossypol content. Similar observations were made in Russia by Smirnova (103), who also reported that the average gossypol content of all varieties of seed of a given species is characteristics of the species. The average content of gossypol was least in the varieties of *G. herbaceum*, intermediate in *G. hirsutum*, and highest in *G. barbadense*. Boatner *et al.* (104) have confirmed the genetic observations. Additional data have been published by Pons and associates (105).

An accomplishment of great value that remains to be made is to demonstrate whether or not gossypol has a metabolic function in the cotton plant and determine whether gossypol-free varieties can be developed by genetic methods. The U.S. Department of Agriculture is reported to be working on the breeding problem and to have encouraging results in early tests (106). The breeding work is based on selection of commercial upland cotton and primitive Hopi varieties with unusually small numbers of pigment glands. (See also Chapter 17.)

V. STORAGE OF RAW MATERIALS AND FINISHED PRODUCTS

1. Fundamental Considerations

The two factors essential to safe storage of oilseeds and small grains are cleanliness and moisture control. High moisture levels and foreign material favor natural respiration of grain and growth of infecting organisms. These biological processes produce heat and may initiate exothermic non-biological reactions which in turn can produce intense heat and cause rapid deterioration of the stored products (107). The practices followed by the cottonseed and soybean industries in controlling these factors have been reviewed by Alderks (108) and Holman (109). The biological processes of the stored products have been reviewed by Altschul (110) and Milner (111). The methods of handling storage of small grains have been reviewed by Oxley (112, 113). Storage techniques have been well developed, and the industries involved

successfully store raw material during each harvest period for an entire year of operation. In fact, material processed late in the year may be superior to early-season products because it is imperative first to process all material that is too wet to be dried for safe storage or exhibits evidence of field damage. Blending programs are followed to avoid exclusive operation on material of the latter type.

TABLE I
INFLUENCE OF MOISTURE CONTENT OF A FEED INGREDIENT ON THE TIME REQUIRED
FOR MOLD GROWTH TO APPEAR AT A STORAGE TEMPERATURE
OF APPROXIMATELY 68°F.^{a,b}

Ingredient	Moisture content	
	(%)	Days
Oats	16.5	19
	15.5	41
	14.0	1300, no growth
Linseed cake	13.0	52
	12.0	1300, no growth
Bone meal	9.5	52
	8.0	1300, no growth
Bran	15.5	32
	14.0	1300, no growth
Hay ^c	15.7	19
	12.9	200

^a Unless otherwise indicated, data are from D. Snow, M. H. G. Crichton, and N. C. Wright, *Ann. Appl. Biol.* **31**, 111 (1944).

^b As assembled by L. R. Richardson and J. V. Halick, *Texas Agr. Expt. Sta. Bull.* **754** (1952).

^c R. Waite, *Ann. Appl. Biol.* **26**, 496 (1939).

a. Feed Moisture Level and Mold Growth

Mold spores are present in all feed products. Their development and growth is the major cause of feed deterioration in storage. Growth of these organisms is critically related to "available moisture" and temperature. The term "available moisture" is used to indicate the fact that the actual moisture level at which mold growth can occur varies with different feed products.

Tables I, II, and III assembled by Richardson and Halick (114) from data published by Snow *et al.* (115, 116), Waite (117), and Davenport *et al.* (118) illustrate how moisture content, temperature,

TABLE II
INFLUENCE OF STORAGE TEMPERATURE ON THE TIME REQUIRED FOR MOLD GROWTH
TO APPEAR ON OATS CONTAINING AMOUNTS OF MOISTURE^{a, b}

Moisture (%)	Days required for molds to appear at:	
	59.9°F.	71.6°F.
18.0	14	10
16.5	22	19
15.5	77	41

^a D. Snow, M. H. G. Crichton, and N. C. Wright, *Ann. Appl. Biol.* **31**, 111 (1944).

^b As assembled by L. R. Richardson and J. V. Halick, *Texas Agr. Expt. Sta. Bull.* **754** (1952).

TABLE III
MOISTURE LEVELS BELOW WHICH FEEDING STUFFS MAY NORMALLY BE SAFE
FROM MOLD GROWTH FOR 3 MONTHS AND 2 TO 3 YEARS^{a, b}

Feeding stuff	Per cent moisture for safe storage for:	
	3 months	2-3 years
Wheat	15.7	14.6
Maize	14.8	13.7
Barley	14.8	13.6
Oats	14.5	13.4
Middlings	14.4	13.1
Bran	14.4	12.8
Soybeans	13.3	11.0
Linseed cake	12.3	11.1
Artificially dried grass	13.7	11.1
Hay	12.6	11.0
Fish meal	11.5	9.9
Meat and bone meal	10.3	8.7
Bone meal	9.5	8.7
Sorghum grain ^c	10-12	

^a Unless otherwise indicated, data are from: D. Snow, M. H. G. Crichton, and N. C. Wright, *Ann. Appl. Biol.* **31**, 111 (1944).

^b As assembled by L. R. Richardson, and J. V. Halick, *Texas Agr. Expt. Sta. Bull.* **754** (1952).

^c M. G. Davenport, J. W. Sorenson, H. G. Johnston, R. A. Hall, and J. Bradshaw, *Texas Agr. Expt. Sta. Progr. Rept.* **1240** (1950).

and length of storage are related to development of mold growth in a variety of feedstuffs.

Richardson and Halick (114) reported that feeds containing molasses were frequently involved in cases of mold and heat damage. They analyzed seventy-five samples of molasses collected through the state of Texas and observed variation in moisture content of molasses rang-

ing from a low of 19% to a high of 36%. In view of the critical relationship of feed moisture content to mold growth, it is essential to control the moisture contributed to mixed feed by molasses.

b. Airtight Storage of Wet Grain

Storage of grains in airtight bins without drying is of interest. In theory, anaerobic conditions should occur and prevent extensive development of the microbiological processes necessary to initiate spontaneous heating. Foster *et al.* (119) have reviewed the literature on this problem and have reported good growth of pigs fed corn which had been stored in sealed bins for two years at 27% moisture content. The corn was dark in color, had a sour odor, and was difficult to handle. Nevertheless, nutritional impairment was not observed in the swine feeding test.

2. Biological Value of Stored Cereal Grains and Soybeans

Assuming proper dry storage of small grains, there is no reason to expect significant deterioration of protein nutritive quality for periods of two years or longer. Mitchell and Beadles (120), for example, observed no change in biological value and true protein digestibility of wheat stored 730 days, a hybrid corn stored 850 days, and a dent corn stored 958 days. Moisture content was in the 6 to 12% range; storage was in sealed tins, free of bacterial or insect infection, at room temperature. This work showed that grains ground to pass through a 1-mm. sieve were equivalent in stability to whole grains.

These authors found soybeans more perishable than cereal grains. Biological value and true digestibility data for protein in meal obtained from stored raw whole beans were irregular and not clearly indicative of storage effects through 404 days. By the end of 1020 days, however, biological value had dropped 13 percentage units and true digestibility had dropped 10 percentage units. The net effect was equivalent to a loss of 28% in conventional protein value (nitrogen \times 6.25). A control soybean oil meal sample prepared from the same beans by the authors' standard method (solvent extraction followed by autoclaving 90 minutes at 17 p.s.i.) exhibited little decrease in protein quality as a result of the entire 1020-day storage period.

The authors suggest that the observed difference in behavior of the grains, raw beans, and heated meal can be attributed to the extent of protease-type enzyme action in these products. The enzymes would render amino acids available for sugar combination reactions (121), and the beans contain 10% of pentose-type sugars which are active in these processes (122). Beans should have favorably high protease

activity, since the viable embryo, in which the enzymes are located, accounts for 92% of the weight of the seed. In the grains, the embryo accounts for less than 10% of the weight of the seed. Enzyme activity in grains would accordingly be less significant.

These observations by Mitchell and Beadles are not in conflict with those of other authors who have shown certain protein solubility changes and even *in vitro* digestibility differences as a result of storage of corn (123), wheat (124), and soybeans (125, 126).

3. Behavior of Gossypol in Storage of Cottonseed, Meal, and Feed Mixtures

The possible effect of seed and meal storage on the biological value of cottonseed protein has not been studied. Interest in this case has been focused on gossypol stability.

Podolskaya (127) reported that during storage of a sample of a variety of *G. hirsutum* the gossypol content decreased from an initial value of 1.15% of the dry weight of the seed to 0.75% after storage of the seed for four months. Boatner *et al.* (104) later examined seventeen varieties of cottonseed in storage at 80°F. for periods up to 300 days. No consistent trend for variation of gossypol content of seed with time was noted in this work. Increasing, decreasing, and relatively constant values were observed. Castillon *et al.* (128) studied three varieties of seed in storage for long periods of time at 38°, 77°, and 85°F. Decrease in gossypol content with time was observed in all cases. The temperature variable did not appear to have a marked effect on the rate of change.

The decrease in gossypol content of stored seed observed by Castillon was accompanied by an increase in content of closely related pigments (diaminogossypol and gossypurpurin). Although these pigments have been differentiated from gossypol by chemical methods, relative physiological activities have not been determined. It is generally assumed that these pigments have physiological effects similar to gossypol, and it is known that the official method for gossypol analysis, in contrast to that used by Castillon, includes diaminogossypol in the gossypol estimate. In view of the interrelation of these variables, and the incomplete information on toxicity of the seed pigments, it cannot be concluded that the toxicity of cottonseed is favorably decreased during commercial storage of seed.

Kupperman and Karon (129) have studied gossypol behavior in storage of cottonseed meal and feed mixtures. Storage of commercial meal samples under conditions simulating normal warehouse practice had little effect on free and total gossypol content. Storage at 140°F., however, caused a marked drop in free and total gossypol over a 90-day

period. When gossypol was added to cottonseed meals and various feed mixtures, there was an apparent loss, or inactivation, of a portion of the gossypol immediately after diet preparation and further apparent loss on storage. This is in agreement with prior work of Olcott (130) and Heywang *et al.* (131), who demonstrated decrease in gossypol toxicity as a result of blending the isolated pigment into feed mixtures. An explanation for this phenomenon is not available. Heywang and Bird (132) assumed that organic amines in the mixture might combine with gossypol and thus cause low analytical values. They were unable, however, to demonstrate inactivation of gossypol as a result of including the following amine sources in chick rations: 0.8% DL-lysine, 1.0% glycine, 0.5% 1,3-diaminopropanol, 0.5% urea, and 20% sardine meal.

Gossypol stability in the practical case of blending cottonseed meal with other feed ingredients has not been studied primarily because of questionable reliability of analytical methods. Kupperman and Karon (129) used a modified *p*-anisidine method of analysis in their work. Storherr and Holley (133) have also studied the problem of analyzing mixed feeds for gossypol and have reported details of an analytical method based on a color reaction of gossypol with phloroglucinol.

Pure gossypol can be safely stored for 18 months at room temperature if protected from light (134).

4. Use of Chemicals for Protection of Stored Grains

Chemical treatments are sometimes used for control of insect infestation of stored grain. The problem in this field is to select substances with low residual toxicity and no interaction with nutrients in the stored products. Powders containing pyrethrum and piperonyl butoxide, a pyrethrum synergist, are frequently used. Dove and Schroeder (135) have recently published data showing very favorable action of oil-free emulsions containing these two chemicals. Fumigation with methyl bromide is another accepted practice (136–138).

Ethylene oxide has been used in the food industry (139) and has been suggested as a sterilizing agent for various products (140). Hawk and Mickelson (141) however, have recently reported inactivation of thiamine and other unexplained effects that restricted rat growth when the ration was treated with ethylene oxide.

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CHAPTER 7

EVALUATION OF PROTEIN QUALITY

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I. INTRODUCTION

Ever since the importance of proteins to life was first recognized, over a hundred years ago, investigators have been attempting to determine the relative values of protein sources as components of diets. Many of the early attempts failed because the basic diets of the time were unsatisfactory, and little real progress was made until the early 1900's.

Looking back on these years through the eyes of Beach (1) and of Vickery (2), one sees a period of rapid development and recognition of essential problems, culminating in the classic work of Willcock and Hopkins (3), and of Osborne and Mendel (4). These two groups demonstrated dramatically that certain amino acids must be present in the diet for normal life to be realized. They also proved that certain proteins are inadequate because they only partly supply the animal's need for particular amino acids. From the early 1920's to 1936, there were numerous unsuccessful attempts to relate the quality of a protein to its amino acid content. In 1936, the isolation in Rose's laboratory (5) of the amino acid that was later called threonine brought this period of uncertainty to a close; it then became possible to replace protein completely with mixtures of pure amino acids.

While this basic work was under way, the need to evaluate proteins became more urgent, and several methods were developed. The usual approach to the problem was to compare the growth rates of rats fed various unevaluated proteins with those of rats fed proteins previously determined to be of good quality. Modifications of this method included determinations of nitrogen balance, digestibility, nitrogen economy in relation to weight gain, and others. Excellent discussions of these and other methods are given by Frost (6) and by Allison (7).

Unfortunately, the chemical complexities of amino acids have made them difficult to isolate, to synthesize, and to resolve. Consequently,

some pure amino acids even now cost \$50 to \$500 per pound, and most investigators have used mixtures of pure amino acids only when there was no natural protein material suitable for their specific problems. As a result, techniques were developed to measure the quality of a *total* protein, rather than to estimate the contributions of various amino acids to the protein portion of the diet. These empirical methods have yielded much information, but they must be regarded now as guides to more discriminating methods of evaluation.

The discussion in this chapter is based on the belief that eventually protein sources will be evaluated in terms of the amounts of available amino acids which they supply. No doubt it will be convenient for some years to use such terms as "biological value," "net protein value," and "protein efficiency ratio"; nevertheless, it seems certain that the value of a protein source will eventually be expressed in at least ten different terms—one for each amino acid in which the nutritionist is interested. Of course, this is already the practice in diet formulation, where the amino acid contents of proteins are often used. These data are, however, still relatively crude for purposes of prediction or of evaluating various methods of processing the proteins.

To illustrate the range of problems in this field, four examples are presented below. These demonstrate clearly that no single figure can be devised to represent *the* value of a protein because proteins are not entities, but collections of various amino acids combined in various ways and present in various concentrations.

New amino acid sources. To study a new or potential source of protein, one determines first its total crude protein content as measured by its nitrogen content, and next its constituent amino acids. The first feeding trials are designed to reveal how well the protein food is tolerated by experimental animals. (Obviously, this aspect of quality is not necessarily related to the amino acid makeup of the protein.) Subsequent feeding tests reveal digestibility and adequacy of the whole amino acid complement for the animals being studied. Finally, data are obtained on the availability of specific amino acids that are critical in the animal's diet.

Effects of processing methods on protein value. The problems here may be illustrated by studies on cottonseed meal, which during oil removal is subjected to variable conditions of temperature, moisture, and cooking time, as well as to differing physical treatment such as rolling and shearing. In the chemical and biological evaluations, suitable criteria must be established, based on expected usage. For example, cottonseed protein that has been subjected to a minimum of heat processing is just barely adequate as a source of lysine for the growing chick and is inadequate for the young turkey, which has a higher requirement. The detrimental effects of prolonged heating on the available lysine are marked; hence, this amino acid is a key to cottonseed meal quality. When cottonseed meal protein is fed in combination with cereal proteins, the problem of the lysine supply of the whole diet becomes even more acute because of the universal deficiency of lysine in grain proteins.

Values of protein concentrates as sources of specific amino acids. Because proteins vary so widely in amino acid content, it is necessary to consider each protein individually in terms of the specific amino acids which it contributes to the diet in significant amounts. Blood meal, for example, is deficient in several amino acids, yet it is a valuable feedstuff because it is a rich source of lysine.

Costs of amino acids from various sources. It is important that amino acids be considered primarily in terms of their use to animals to allow normal growth, reproduction, and long life. But amino acid supplies are expensive in all parts of the world, so it is also important that they be used economically. We must, therefore, consider the cost of a given amount of a needed amino acid. Is this most economically provided in synthetic form, or will a less potent source provide a cheaper supply? As we shall see later, the problem of estimating requirements for amino acids is logically the economic problem of determining the cost of the amino acids relative to the value returned.

In the present chapter we attempt to evaluate critically the various biological and chemical methods in use, and to propose some objectives for future research. The first part of the discussion (Section II, by C. R. G.) deals with methods that rely on responses of mammals and birds to variation in amino acid supply. We have tried to present a general discussion while referring specifically to methods for particular kinds and ages of animals. For more detailed accounts, the reviews of Frost (6), Allison (7), and Duckworth (8) are excellent. In the next part (Section III, by R. W. C.) we present some of the more important chemical and microbiological methods, all of which must, of course, ultimately be evaluated by the use of animals. These chemical methods have proved helpful in past research and development, and, as new techniques are developed, chemical methods will become even more important in the establishment of commercial standards.

It will be evident to those interested in ruminant nutrition that we have completely bypassed this field. The values of protein foods for ruminants cannot be related clearly to their values for simple-stomached animals, because in these animals the ingested food is utilized by rumen microorganisms, which are in turn digested and metabolized by the host.

II. DIRECT METHODS USING BIRDS AND MAMMALS

1. Determining Amino Acid Requirements

a. The Problem of Establishing Valid Criteria

Estimates of protein quality must ultimately be based on a product's ability to satisfy the amino acid needs of animals. These needs themselves are difficult to determine with accuracy because there are, as yet, no precise standards for their estimation.

The problems of assessing requirements for amino acids are not fundamentally different from those encountered in a study of the needs

for other nutrients. Presumably, one should be able to feed various levels of an amino acid and determine the response in growth, maintenance of nitrogen equilibrium, egg production, or some similar amino acid-demanding function. Many such experiments have been performed, but as yet nutritionists have not developed universally acceptable criteria for measuring requirements quantitatively.

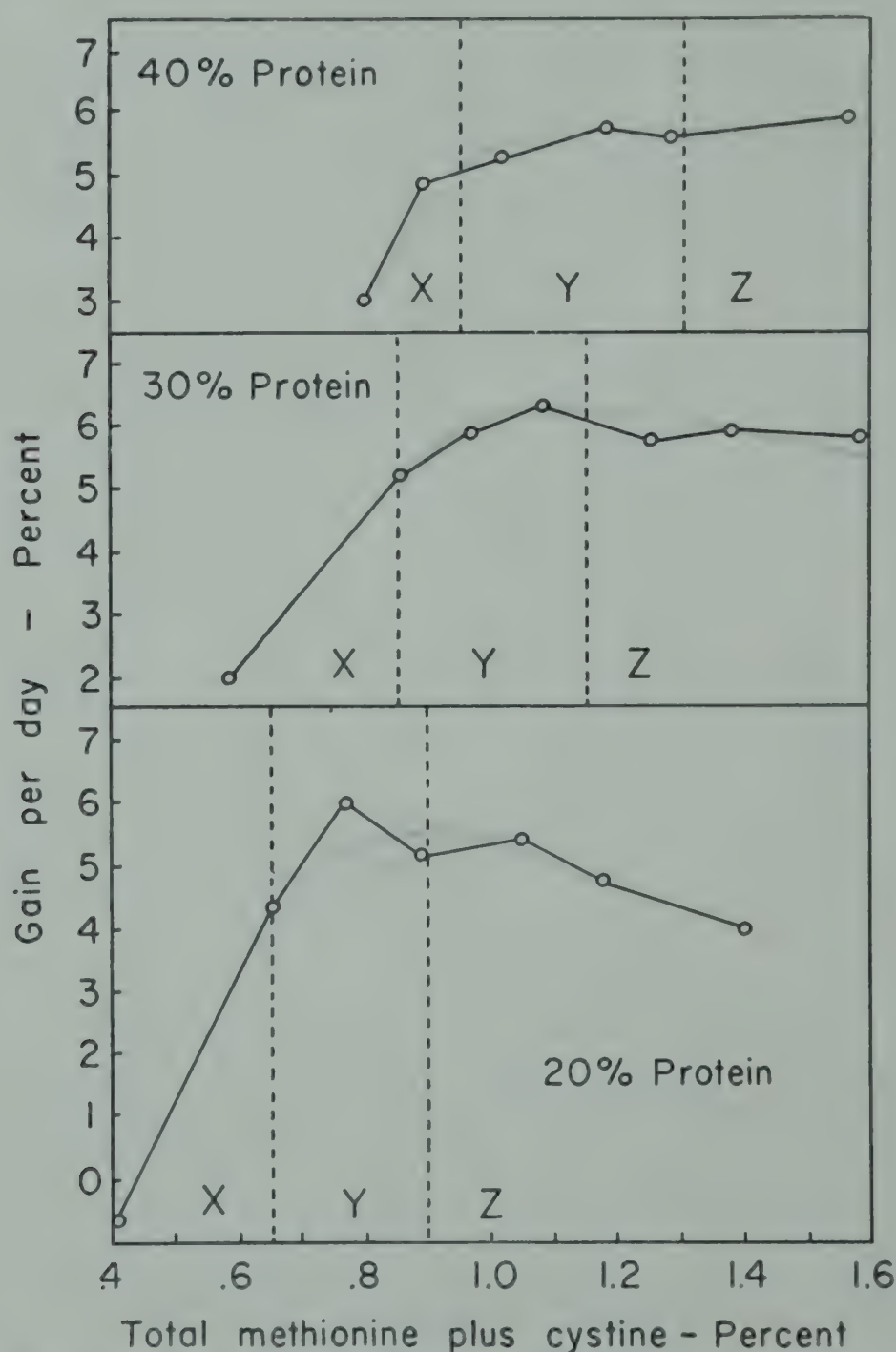


FIG. 1. Growth rates of chicks fed three protein levels and various levels of methionine plus cystine. See text for details.

In order to illustrate some problems of determining amino acid requirements, three examples of concentration-response curves are presented in Fig. 1 (9).

Here the concentrations of total methionine plus cystine are plotted against the growth rates of chicks that were 10 days old at the start of an 8-day experiment. The basal diet was composed of purified materials and contained an isolated soybean protein that was very low in cystine content and moderately low in methionine. Each point represents the average growth rate of ten chicks

fed either the basal diet (the lowest amino acid concentration) or this diet supplemented with methionine. Given such diets, the chick uses some of the methionine as such, and transforms some of it into cystine for tissue synthesis. These data are typical of the sort that have been used to provide estimates of amino acid requirements.

The data of Fig. 1 were used originally to show that the requirement (expressed as per cent of the diet) increases as the protein level is increased, a relationship that is discussed later. Three curves are given here to illustrate the effect of protein level on the shapes of response curves and to point out the problem of determining at what amino acid levels the requirements are satisfied. The points have been connected arbitrarily by straight lines, but this is done with the recognition that the curve should be continuous. Also, the

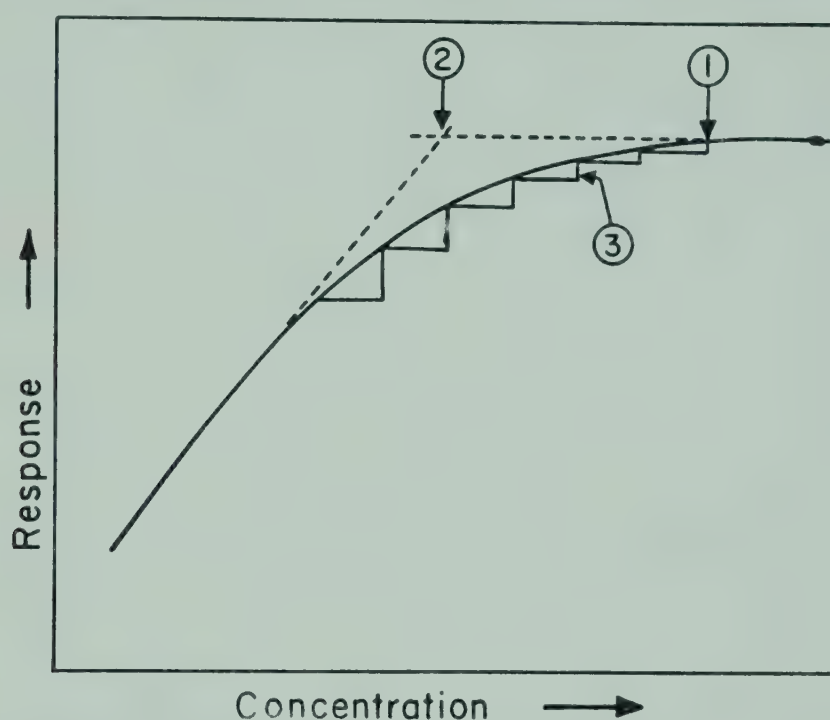


FIG. 2. Idealized response curve for an essential amino acid, illustrating three criteria for establishing the requirement. See text for details.

plots have been divided into three convenient areas: In area X, the requirement has certainly not been met, whereas in area Z, the minimum level has undoubtedly been exceeded; we are interested in the middle area Y, where the response curve changes from an ascending one to a descending or level one.

At the 20% protein level, methionine supplementation showed a marked effect on growth up to concentrations of 0.7 or 0.8% total methionine plus cystine. As the methionine concentration was raised above that level, the growth rate definitely declined. Raising the protein level to 30% resulted in a similar need for methionine supplementation, but, at this level of protein, excess methionine did not have a depressing effect on growth. At the higher protein levels it is more difficult to estimate the requirement.

Some of the criteria that may be used to establish the amino acid requirements are presented graphically in Fig. 2, where the numbers refer to the following three methods:

(1) *Determine the minimum amino acid level which gives a response not significantly different from the maximum attainable.* This method has been discussed by Hegsted (10), who pointed out that: "This is at best a highly

uneconomical procedure for, in effect, one discards the major portion of his data in making the test and uses the data from only two small groups at exactly the point where large numbers are required to prove small differences significant."

(2) *Determine the point of intersection of straight lines of positive slope and of zero or negative slope.* These lines may be drawn by inspection (11) or may be calculated (12), using the amino acid concentration or the logarithm (13) of the amino acid concentration. This way of assessing the requirement is satisfactory in many ways, but it is definitely arbitrary, because one must decide which points to include in each of the two curves.

(3) *Determine the concentration necessary for maximum economy.* For practical application of data, this is probably the best criterion, because the results are expressed in terms that require least interpretation and fewest value judgments. The results, however, are valuable only for the particular conditions of the test. When the added amino acid is in a relatively pure form, the calculations are relatively simple, but with some modifications the same principle can also be applied to amino acid concentrates.

The first and second criteria can be easily understood from Fig. 2, but the third may require further comment. As the concentration of amino acid is increased (at some fixed cost per increment), the increment of response decreases to zero. Because proteins, and especially single amino acids, are relatively expensive, there is a definite possibility that maximum return from amino acid supplementation may be realized at a level somewhat lower than that which allows maximum growth.

Any of several criteria may be used successfully as long as we recognize that the standard is an arbitrary one. The methods employed usually do not allow estimation of requirements to more than two significant figures.

It must also be realized that these estimations are themselves affected by variations in analytical data of amino acid contents of proteins. The most common method of determining an amino acid requirement is to feed a diet of known amino acid content (established by analysis of the protein), and determine the response to added amounts of a pure amino acid. Errors in the basic amino acid content figure may thus magnify errors in the subsequent biological determinations.

b. Methods for Estimating Requirements

Animals in general lack the ability to synthesize certain amino acids. Rose (14) has termed these the *essential* amino acids because they must be present in the diet for normal growth to be maintained. If the quantity of an indispensable amino acid supplied in the diet is not sufficient to allow a maximum rate of growth, the appetite is curtailed (11) and the total synthesis of body protein is reduced, but each of the dif-

ferent tissue proteins formed under these conditions remains constant in its characteristic amino acid composition (15, 16).

(1) *Growth*. Tissue protein increments are closely dependent on dietary supply of amino acids, and growth is, therefore, usually a good criterion of adequacy of amino acids in a diet. When growth is the criterion, the assumption is made that the composition of the body is not greatly affected by varying the amino acid content of the diet. This assumption is valid for amino acid contents (15, 16), but water and fat contents are markedly affected by the protein nutrition of the animal. In general, when the diet is deficient in the total amino acid complement (i.e., dietary protein) in relation to dietary energy supply, more fat is deposited than when the diet is adequate in protein; when the diet is high in protein, even less fat is formed (17). A deficiency of a single amino acid, however, results in a lean carcass (18). In all these situations the amino acid contents of the tissues remain quite constant, but water and fat replace one another.

Following Rose's work (14, 19), mixtures of pure amino acids have been used for the quantitative estimation of the amino acid requirements of various animals. The question of the validity of these estimates has often arisen, especially when the maximum growth that was obtained with the supplemented mixtures was below that generally considered to be optimum. For chicks, however, the discrepancies in estimates are not very large (11).

(2) *Carcass analyses*. One method of estimating the proportions of the various amino acids that are required is to determine the absolute or relative amounts of the several amino acids in muscle, in other tissues, or in whole animals (20). Estimates on this basis assume an equal efficiency of utilization of dietary amino acids, a constant metabolic pool size, and an equal use of all amino acids for purposes other than body protein formation (e.g., for hormone synthesis). Perhaps the strongest argument against the general value of carcass analysis data for estimation of requirement lies in the fact that animals are able to synthesize about half of the amino acids needed for tissue formation, and this group of amino acids represents about half of the total amount of the amino acids needed (19).

(3) *Nitrogen balance*. Most tissues of the body are constantly changing, being broken down, then built up again. More precisely, their constituent amino acids are in a dynamic state (21), with some being released and others becoming incorporated into proteins. As the body proteins are broken down, nitrogen is lost in the urine and feces. A certain amino acid intake is required to maintain a state of equilibrium or nitrogen balance, and, unless the animal ingests as much nitrogen as it

excretes, it will be in a state of negative nitrogen balance. The amino acid intake required to maintain this state of equilibrium can be determined by measuring the amount of nitrogen in the diet and the amount excreted (7). Workers using this basic technique and many modifications thereof have provided much valuable information on various proteins as foods, as Allison (7) has outlined so ably. The experimental animals most frequently used have been rats, dogs, and man.

(4) *Reproduction*. During pregnancy, lactation, or egg production, the food needs of the mother are increased, and this greater demand may be taken as a measure of the quality of the food. The daily needs for amino acids are increased with the pressure of reproduction, but other nutrient needs are also increased; hence an increased food intake is the main response of the mother. The amounts of amino acids that are incorporated into eggs by a hen laying 300 eggs per year are surprisingly high, of the order of 2 kilograms per year. The rate of production of eggs (22) and egg weight (23) are good criteria for establishing the amino acid requirements of the laying hen. The amino acid content of eggs is not influenced by the amino acid content of the diet (24). (See also page 162.)

c. Tables of Amino Acid Requirements

There will undoubtedly be continuing controversy about the validity of various figures for the amino acid requirements. Nevertheless, much of the present information can be applied to practical problems, despite its semiquantitative status. The animals for which we have the best estimates of requirements are those which are of economic importance, notably chickens, turkeys, and swine. (See Chapters 2 and 13.)

2. Factors That Affect the Amino Acid Requirement

Amino acid requirements can be expressed in several ways, such as milligrams of amino acid per kilogram of body weight; milligrams of amino acid per Calorie consumed; per cent amino acid in the total diet; or per cent amino acid in the protein. As more is learned about the interrelationships among various nutrients, this expression will probably become more complex. Yet we already have available considerable information about some of the factors that influence the requirements.

a. Growth

It has long been known that the young, growing animal requires a higher protein level in its diet than does the mature animal. Since the protein need is a summation of amino acid needs, it is possible that rapid growth imposes a need for a higher dietary level of amino acids than does slower growth. There are relatively few data on this exact

point, however. Edwards *et al.* (25) have reported that for maximum growth their fast-growing birds require 1.1% lysine in a diet containing 20.5% protein, but their slower-growing White Leghorns require not more than 1.0% for maximum growth. Bird (26) found the lysine requirement of 8-week-old chickens to be the same as that of very young birds, when both estimates were based on the lysine content of the protein. As animals mature and their growth rates approach zero, the maintenance requirements for total protein become very low, but the amino acid requirements, expressed as per cent of the protein, do not necessarily decrease. In rats, keratin synthesis for normal hair growth becomes a major factor in influencing amino acid needs, particularly those for methionine and cystine (27).

b. Protein Level

There is abundant evidence that all requirement for a particular amino acid is significantly influenced by the levels of the other amino acids present in the diet. Such an interrelationship was first demonstrated with the lysine needs of chicks (28), but has since been extended to other amino acids and to other animals. For a time there was some disagreement among laboratories as to the extent to which the requirement is affected (11). This may seem a trivial point, but if one hypothesis were true, namely that the requirements increase in direct proportion to the protein level, then a slight amino acid deficiency would never be overcome by feeding a higher protein level. Studies with mice (29) showed that the requirement for lysine can be met by feeding high levels of gluten, a protein low in lysine. The evidence is accumulating (7, 30) that, as the protein level is raised, the amino acid requirement also increases, but at a lower rate; consequently, a partial deficiency can be overcome by feeding a higher level of the protein. This method of satisfying requirements is inefficient for general protein economy, but it offers advantages when there are locally abundant supplies of inexpensive proteins that are slightly deficient in an amino acid that is not readily available from other sources.

An important corollary is that care must be taken in amino acid bioassays to be sure that correction is made for the addition of proteinaceous material other than the amino acid being studied (31).

c. Energy Level

The major factor that determines food intake under ad libitum conditions is the energy need (17), and this in turn exerts a great effect on amino acid needs. A ration that is low in energy will be consumed in larger amounts than one that is energy-rich; hence, if the ratio of protein to other components is constant, there will be a higher

amino acid consumption per day, and per Calorie consumed. A detailed discussion of this has been included in Allison's excellent review (7). (See also Chapter 13.)

There are relatively few data on the relation of amino acid requirements to dietary energy levels, but it is known that the energy level of the diet appears to affect the lysine requirement of the chick (32), and also its methionine requirement (33). This effect is undoubtedly not specific for these amino acids alone, however, but merely reflects differences in basic needs for energy and for amino acids, with the first related primarily to body surface and the second to the mass of metabolically active tissues.

d. Reproduction

One of the greatest demands for dietary nutrients exists during reproductive phases in the life of the female. These needs are especially great in animals that are kept primarily for providing man's diet with materials related to the reproductive processes, such as milk and eggs.

One fact that is often overlooked in a discussion of amino acid needs during reproduction, however, is that, while amino acid needs increase (34), energy needs increase as much or even more, and, since the animal eats to satisfy this energy need, its total food intake increases markedly, and its amino acid intake also increases.

e. Other Nutrients That Affect Amino Acid Requirements

We now know that a number of vitamins or other nutrients markedly affect amino acid utilization. Perhaps the best illustration of one kind of interrelationship is the effect of niacin on the tryptophan requirement. This has been studied extensively in the ten years since the discovery (35) that a dietary deficiency of niacin imposes a greater need for tryptophan, thus indicating that tryptophan can be converted to niacin.

A different type of interrelationship is seen between pyridoxine and tryptophan. Here the vitamin pyridoxine is necessary for the normal metabolism of tryptophan (36). There are undoubtedly many similar relationships that have not yet been uncovered. The investigator must always be on guard against unintentional introduction of effects of other nutrients on amino acid metabolism.

3. The Animal's Use of Amino Acids in Protein Combination

a. Availability of Amino Acids

It is often convenient to refer to the amounts of available amino acids present in protein foods. By this term we mean the amount of

each amino acid that is present in a form that is ultimately usable by an animal. Of the *total* amount of each amino acid present, part is *digestible* and hence absorbed. Part of this absorbed material is *available* for satisfying the amino acid needs of the animal. There are a number of reasons for the differences between the amounts of *total* and *available* amino acids in a protein. Before the proteins become foods, they are functional units of living tissues, and, as such, they may be protected against attack by the degradative mechanism of the digestive tract. Also, some plant materials contain enzyme inhibitors (37, 38). Damage by heat during processing has been clearly shown to decrease total protein digestibility (39). (See also Chapter 5.) If the linkages that exist in the ingested protein cannot be hydrolyzed by the enzymes present in the digestive tract, the undigested or partially digested residue will be lost in the feces, or will be used as food by the microorganisms of the lower part of the alimentary canal instead of by the animal itself. (See also discussion on page 175.)

Most of the available evidence concerning the nature of the process of enzymic degradation of protein by animal proteinases indicates that an "explosive" type of breakdown occurs, and that each protein molecule attacked is rapidly degraded to its ultimate peptide or amino acid products (40). Thus, as hydrolysis proceeds in a digestion mixture, the average molecular size of the end products tends to remain the same. As a result, the relative proportions of the various amino acids simultaneously available in free or small peptide form for absorption into the portal circulation (41) of a non-ruminant animal are probably usually similar to those occurring in the dietary protein.

Almquist (42) has called attention to the existence of interesting relationships between the relative abundance of different amino acids in the crude protein of the diet, the proportionate needs for these amino acids by the animal receiving the diet, and the observed levels of various amino acids in free form in the animal's blood. It seems possible that these findings are of considerable fundamental importance, since they suggest a basis for development of a comparatively simple and rapid means of direct identification of the amino acid or acids in a diet. A related method of investigation of protein quality involving amino acid analysis of the urine from animals fed different protein sources has been investigated (43) and has been found to be of limited value, except possibly in mice.

Attempts have been made to study the biological availability of particular amino acids in different protein sources by determination of the percentage of an amino acid ingested as dietary protein that is absorbed by the animal, as measured by residues in the feces. Studies of

this type generally show that the amino acids of eggs, milk, and meats are more available than those of cereal and legume foods (44), and thus they are in agreement with the findings of conventional protein digestibility studies. A relatively low availability of the lysine and a high availability of the arginine of cottonseed meal has been observed by this technique (45).

The distribution of the various amino acids in feces, however, is probably influenced not only by the composition and absorption of dietary amino acids, but also by activities of the intestinal microflora. That considerable losses are possible is suggested by the finding with protein-depleted rats fed raw soybeans that about 70% of the lysine and 50% of the methionine was neither excreted in feces nor absorbed in utilizable form by the animals (44). Similarly, the disproportionately large quantities of cystine present in contents of the small intestine of rats fed raw soybean meal were found to disappear, presumably as a result of putrefactive activity in the bowel, during passage of the diet residue through the animal's large intestine (46). These difficulties can be avoided by analysis of digestion mixtures; however, this method of investigation of amino acid availability is technically difficult, necessitates killing the animal, and, although it has the advantage that results are probably less subject to influence by activities of intestinal microflora than those obtained by analyses of feces, there is reason to believe that the animal's own secretory activity may distort the findings. Indeed, it has been observed that protein secretion may cause the anomalous finding of "negative" coefficients of digestibility in parts of the upper intestinal tract (47). Also, it has been reported (48a) that the digestion mixture obtained from the small intestine of a dog fed the lysine-free protein zein contained as much lysine as that from an animal fed the lysine-rich protein lactalbumin. Lyman (48b) has found that feeding raw soybeans to rats causes depletion of pancreas nitrogen by enzyme secretion, and this in turn causes apparently poor utilization of raw soybean protein.

b. Imbalance of Amino Acids

For efficient amino acid utilization it is necessary not only that proteins be digestible but that the relative amounts of the various amino acids be not too far from the optimum. Harper (49) has suggested some criteria to distinguish among the terms *imbalance*, *toxicity*, and *antagonism*. Whether or not these terms can conveniently be differentiated cannot be argued here. As Harper points out, however, it has been known for many years that high levels of some individual amino acids are not well tolerated, and that in some instances the adverse effects of

such excesses can be overcome by adding a specific amino acid or vitamin. A nice example is the observation that high dietary levels of leucine exert adverse effects which can be overcome by increasing the isoleucine level of the diet (50). Thus the apparent isoleucine requirement may be influenced by the dietary leucine level. In some cases the effects are not specific but may be overcome by the addition of a mixture of amino acids (i.e., protein) (9). High levels of some amino acids cause an increase in the dietary needs for niacin, in a way that may not be related to the niacin-tryptophan interrelation discussed earlier. There are numerous instances in the literature of poor proteins that cannot be made entirely satisfactory even by extensive supplementation with amino acids, such as by burned fish meal (51); corn gluten meal (52); gelatin (53); and blood meal (54). (See also Chapter 13.)

c. Time Factors

The work of Cannon (55) and of Geiger (56) has shown clearly that amino acid supplements must be given at about the same time as the rest of the diet, and that a delay of even two hours can result in poor utilization of all amino acids. Tracer studies using normal or deficient test meals with mice that were previously fed either phenylalanine-deficient or normal diets showed that in amino acid utilization the composition of the meal was much more important than previous dietary history (57).

4. Biological Methods of Estimating the Adequacy of Proteins

a. With Growing Animals

In the discussion on the determination of amino acid requirements, it was pointed out that growing animals are sensitive to variations in the levels of essential amino acids. This effect on growth was recognized in most of the early studies (1, 2) and is still probably the most widely used measurement of protein adequacy.

(1) *Growth rate*. There are many different ways of using growth rates as measures of protein adequacy, each with some merits, but also subject to definite limitations. One of the earliest methods developed was that of Osborne and Mendel (4), who fed rats low-protein diets (10%) in which most of the protein was provided by some particular protein source. Later they modified their methods somewhat and expressed their results in *protein efficiency ratios* (see below).

In most of the work with chickens and turkeys, growth rates have been taken as criteria of amino acid adequacy. This work has been

reviewed in detail by Almquist (11) and others (7), and perhaps we need say only that this method has yielded data of great value in applied nutrition.

(2) *Protein efficiency ratio*. This is the weight gain of an animal per weight of protein consumed, under certain specified conditions: viz., a dietary protein level of 10% fed ad libitum to young rats. The data obtained with this method are quite reproducible within a laboratory and have proved helpful in protein evaluation. Hegsted and Worcester (58) have shown clearly, however, that protein efficiency is a function of weight gain rather than a characteristic of the protein fed, and hence little additional information is obtained by using protein efficiency ratios rather than weight gains. One major disadvantage is that the results are a measure of a particular diet, not of a particular protein. This is dramatically demonstrated by adding a supplement of a needed amino acid to a diet producing a low ratio: the result is a marked increase in the ratio. A further difficulty is that, with 10% protein, even *maximum* ratios probably do not represent *optimum* protein value, because the requirements for various amino acids at a 10% level are lower than those at a level of 18 or 20% protein. Within these limitations, however, the method has proved valuable.

(3) *Gross protein value*. The method here is similar to that for determining the protein efficiency ratio. A protein is fed as a supplement to cereal proteins and is compared to casein, which serves as a standard. This method, too, has yielded useful results, particularly in the hands of Heiman *et al.* (59) and of Carpenter *et al.* (60). Its value lies in its practical applications because protein concentrates are generally used to supplement cereal proteins. Its limitations are (1) that it gives a value that can be compared with other proteins only in supplementary terms, (2) that the standard used (casein) is not very reproducible and is not satisfactory for chicks because it is deficient in glycine, cystine, and especially arginine, and (3) it is principally a measure of the available lysine supply. The results are expressed in grams of gain per gram of test protein consumed in relation to a similar figure for casein.

(4) *Biological value*. This is defined as the percentage of absorbed nitrogen that is retained by the body as determined by nitrogen balance methods. The staunchest advocate of the method is Mitchell (61), in whose hands the complex and laborious procedures are quite reproducible. The technique is rigidly defined by Mitchell for growing rats, based on the original method of Thomas. For details the reader is referred to Mitchell (61), Allison (7), and Frost (6).

Measurements of biological values provide a comparison of various

proteins as the only amino acid source, but their usefulness is limited for several reasons: (1) They are determined at low total protein levels, but the amino acid requirements at adequate protein levels are probably not directly proportional to those at inadequate protein levels. (2) Since no attempt is made to determine the values for amino acid components, a deficiency of one amino acid will cause an apparently low biological value. Thus the addition of a small amount of one amino acid may raise the apparent biological value from a low figure to a high one. (3) Since a single protein is almost never the sole amino acid source in diet formulation for human beings or for farm animals, the application of biological value data to applied nutrition is difficult. Two proteins, each with a low biological value, may together produce a mixture with a high value.

b. With Mature Animals

Most of these methods involve estimation of nitrogen balance and may be expressed as the biological value (61) or as the *nitrogen balance index* of Allison (7), which has been discussed by Frost (6), and which is the relation of absorbed nitrogen to nitrogen balance. Cannon (62, 63) developed a method of measuring the return of protein to tissues of protein-depleted animals in relation to the quality of protein fed during the repletion period. This method has the advantage of rapid assay, re-use of experimental animals, and independent evaluation for repletion of several tissues. The validity of the data obtained by this technique has been questioned by Duckworth (8). Recent collaborative tests (64) comparing the protein-repletion methods with growth methods fail to reveal any marked advantages of the repletion methods over simple growth methods.

5. Use of Mammals and Birds in Bioassays for Amino Acids

It is the value of a protein as a source of the various amino acids which is most important. The various bioassay methods of evaluating total protein are in reality only measures of the whole amino acid complex. Some of the methods (such as the determination of gross protein value) emphasize the lysine content of a protein (60), but thus far no satisfactory method has been developed of determining quantitatively the availability of the individual essential amino acids.

Comparison of methionine bioassays and chemical determination (65) led to the development of techniques in which a diet deficient in a single amino acid is supplemented with low levels of various proteins or with graded levels of amino acids, and from a typical response curve the available amino acid content is estimated. The best results thus

far have been from studies of the lysine content of blood meals (31, 66), but other protein sources have also been studied in this manner (67, 68). When such bioassay techniques are used, especially with materials that are relatively low in content of the amino acid being studied, a significant error may result from the fact that the amino acid requirement increases as the protein level is increased (28).

III. INDIRECT EVALUATION OF PROTEIN QUALITY—BIOCHEMICAL METHODS

1. Introduction

Simple and appropriate biochemical tests for evaluation of quality of the proteins of various foodstuffs would be of obvious value in promoting sensible trading in protein sources. By facilitating rapid evaluation of progress in improvement of nutritive quality they also would be valuable to commercial processors of protein foods. The practice of considering protein as a single dietary component and combining, under the term "protein quality," all the many factors that influence the degree of correspondence of an animal's amino acid requirements with the capacities of different protein sources to supply them tends to give a misleading appearance of simplicity to the problem of development of such indirect, biochemical methods.

The nutritional value of an individual ingredient in a diet is often of importance only to the extent that it influences the amino acid adequacy of the complete mixture that is to be fed. Thus the actual worth of a protein-contributing ingredient may at times depend on the ability of the ingredient to supply a relative excess of a particular amino acid in which the diet is deficient. As cited previously, blood meal gives poor growth when fed as the only source of protein in the diet of chicks because of its deficiency in isoleucine (54) and is, in a sense, of inferior protein quality. But because of the relatively large amounts of lysine in blood proteins (69), addition of blood meal to an otherwise lysine-deficient diet may greatly improve its nutritional quality. Although results of chemical determinations of isoleucine content of blood meal samples might be expected to show correlation with findings of performance tests in which such meals are tested as sole sources of protein, the criterion of isoleucine content would be of no value to a feed manufacturer who intends to use this material to increase the lysine content of his products.

It seems evident that the development of biochemical methods of evaluation of the nutritive value of protein which are of sufficient flexibility and scope to have general practical utility is really dependent on advances in understanding of the specific amino acid needs of ani-

mals, improvements in methods of amino acid analysis, and progress in detection and measurement of factors that influence the physiological yields of the amino acids contained in foods. As new information and techniques have been developed, various *partial* solutions to this problem of indirect, biochemical evaluation of protein quality have been suggested. Some of these will be considered here.

2. Prediction Based on Analyses of Amino Acid Composition

a. Chemical Score

In 1946, Mitchell and Block (70, 71) reported the results of a study of the relationships between amino acid composition and biological value of a large number of protein foods. For purposes of comparison, they assumed that whole egg contains, per unit of total nitrogen, an adequate quantity (but no excess) of each of the amino acids which are required preformed in the diet of the growing rat. They then compared the level of each of the amino acids present in the protein being studied with those present in the proteins of the whole egg. By this method, a "chemical score" was calculated which represented the degree of deficiency of the protein food in the limiting amino acid. The chemical score was equal to 100 minus the greatest percentage deficit in content of an essential amino acid. Thus, for the proteins of corn, there was a 72% deficit of lysine, making the chemical score for this protein source $100 - 72$, or 28. Chemical scores for the protein materials studied by Mitchell and Block were shown to be correlated with the corresponding biological values determined by using the nitrogen balance method with rats.

Since the reporting of the original evaluation method of Mitchell and Block, somewhat similar calculation procedures have been suggested by Kühnau (72) and by Oser (73). Mitchell has chosen what he feels to be certain desirable features of each of the above procedures for calculation of a predicted value for quality and has incorporated these into a "modified essential amino acid index method" (74).

b. Limitations of Chemical Scores

The above-mentioned index methods of prediction of quality have been criticized (6, 8, 75) on the basis of uncertainties concerning the accuracy of the analytical values for amino acid composition. Moreover, the yields of indispensable amino acids to an animal by a particular food are apparently not always solely dependent on the concentrations of these amino acids in the food. It does seem clear, however, that composition is at least an important qualifying factor in determining

physiological yield. Hence the aim of approaching the problem of quality evaluation by comparisons of the degree of correspondence between amino acid composition and animal needs seems to have the important advantage of being theoretically sound. It is also apparent that the employment of a "standard" protein such as egg or some other food as a material representing nutritionally ideal proportions of amino acids is only temporarily justifiable because adequate information on the amino acid requirements of different animals is not yet available.

3. Empirical Physical and Chemical Methods

It has been observed that differences in biological quality of the proteins of foods brought about by processing are often accompanied by changes in solubility or chemical properties of the protein components. It is usually uncertain whether or not these changes are actually responsible for the difference in biological utilization. It often appears that the relationship is only distant and hence is unreliable as a basis for evaluation. The physicochemical methods for evaluation of protein quality based on such observations are, for the most part, empirical; correlation with results of animal tests has been obtained only by careful selection or arbitrary adjustment of the conditions of *in vitro* testing. The necessity for strict adherence to such arbitrarily selected conditions imposes limitations on the general applicability of the methods. Thus, a satisfactory method of evaluation of quality for solvent-extracted soybean meals may be found unreliable when applied to cottonseed meals, or even to screw-pressed soybean meals. Nevertheless, a number of interesting approaches have been made to the development of useful physicochemical methods of testing for quality of protein, and these have been of value in certain situations.

a. Chemical Index

One of the earliest and most successful methods of chemical evaluation of protein sources was described by Almquist *et al.* (76). Their procedure involved analysis of animal protein concentrates for intact protein, protein decomposition products, pepsin-indigestible protein, and protein soluble in hot water. The relative quantities of these were found to bear predictable relationships to the quality of protein concentrates as determined by chick growth studies. By a trial-and-error process, an expression best describing the relationship of analytical results to chick growth was found to be

$$\text{Protein quality index} = A - (B + 0.6C) + 0.4D$$

where A = percentage of total nitrogen precipitated by copper ion in basic solution (includes B and C).

B = percentage of total nitrogen not solubilized by peptic digestion.

C = percentage of total nitrogen soluble in hot water (gelatin fraction).

D = percentage of total nitrogen precipitated by phosphotungstic acid.

The relative weighting of the values of the various nitrogen fractions in the equation is based on the assumptions that the intact, pepsin-digestible protein fraction is of greatest and constant nutritional value; that the gelatin and the phosphotungstic acid-precipitable fractions have only about 40% of the value of the intact, copper-precipitable protein; and that the pepsin-resistant fraction is without nutritional value. Reasonably good agreement between results obtained by this chemical method and by actual feeding tests has been reported for a number of animal-protein concentrates (77, 78) and some protein materials of plant origin (79, 80).

A procedure similar to that of Almquist *et al.* (76) for estimating quality of animal-protein concentrates involves determination of the quantity of pepsin-indigestible protein in the sample (81). In addition, the method includes steps designed to identify the various components making up the indigestible fraction. Reproducibility of the method is claimed to be good, and data are given supporting the authors' contention that the pepsin-indigestible fractions of protein concentrates are of negligible nutritional value.

b. Nitrogen Solubility (See also Chapters 5, 14, and 17.)

(1) *Soybean protein.* Evans and St. John (80) studied the changes in solubility of protein fractions of solvent-extracted soybean meal which result from wet heat treatment. By the method of Lund and Sandstrom (82), the proteins of soybean meal samples were fractionated into albumins (water-soluble fraction), globulins (fraction soluble in 5% potassium chloride), prolamins (fraction soluble in 70% ethanol), glutelins (fraction soluble in 0.2% potassium hydroxide), and residual protein (fraction not dispersible in any solvent used). Their data (see Table I) show the observed changes in solubility resulting from progressive increases in severity of heating. It is evident that moderate heating tends to reduce the quantity of "albumin" and that much of this fraction appears to be displaced into "residual protein" and "glutelin" fractions. Heating of greater severity appears to interfere seriously with the dispersibility of the proteins in alkali as evi-

denced by progressive reduction in quantity of "glutelin" and increase in "residual protein." Thus, the effect of heat treatment of raw soybean meal on its content of alkali-soluble protein seems to parallel roughly its action on protein quality of soybeans: i.e., moderate heating increases, and severe heating decreases. These authors studied various commercial samples of soybean meal by the protein-fractionation technique and evaluated them in chick feeding tests; they report general parallelism between quality and glutelin content. The apparent persistence of a water-soluble, heat-stable "albumin" component of soy is probably related to the observation that from 4 to 8% of the total nitrogen of fat-free soybean meals is non-protein (83).

TABLE I^a
EFFECT OF WET-HEAT TREATMENT ON PROTEIN FRACTIONS OF
SOLVENT-EXTRACTED SOYBEAN MEAL

Interval of autoclaving at 121°	Distribution of total protein				
	Albumins	Globulins	Prolamins	Glutelins	Residual protein
min.	%	%	%	%	%
0	75.6	5.7	3.5	6.0	9.2
5	49.6	6.3	2.7	19.8	21.6
15	8.8	6.4	3.4	45.8	35.9
30	6.4	1.6	3.2	38.6	49.6
60	7.5	1.2	2.6	22.6	65.4
120	8.6	1.0	2.7	10.0	77.3

^a Taken from data of R. J. Evans and J. L. St. John, *J. Nutrition* **30**, 209 (1945).

Simon and Melnick (84) studied the relationships between water-soluble nitrogen, urease, and antitryptic activities, and growth-promoting value of different types of soybean products. Their data, like those of Evans and St. John (80), show the reduction in water-extractable protein that is ordinarily associated with the biological improvement of soybeans by heat treatment.

A relatively simple procedure for evaluation of soy products according to their contents of water-extractable protein has been described by Loska and Melnick (85). Balloun *et al.* (86) also reported relationships between water-soluble protein content, urease activity, and protein quality of soybeans.

Whether the marked reduction in water extractability of soy nitrogen by heating is a primary effect of denaturation of all protein components is not clear. There is evidence that the water dispersibility of the principal globulins of soy is not reduced if they are heated after

solution but that they appear to become increasingly non-dispersible when heated in the presence of other soy fractions (87). There is considerable evidence that the partition of nitrogen obtained by such procedures is quite arbitrary. By electrophoretic studies of the "globulin" fraction of soybeans, it has been shown that yields and degrees of homogeneity of preparations are sensitive to changes in methods of fraction preparation (88).

(2) *Cottonseed protein*. Several investigators have studied the relationship of protein quality to nitrogen solubility in cottonseed meals. The complicating effects of gossypol toxicity in determining cottonseed meal quality is discussed in detail elsewhere. (See Chapter 17.) There is a general tendency for heat treatment of cottonseed meal to reduce the ease of water dispersibility of the meal proteins. Olcott and Fontaine (89) concluded that the nutritive values of different cottonseed meal samples appeared to vary inversely with the degree of denaturation of the cottonseed proteins as estimated by extraction of the meal sample with a 3% sodium chloride solution. Lyman *et al.* (90) did not find this effect. These workers suggested an improved method which was designed to take into account both nitrogen solubility and total gossypol content. Their chemical index is defined as per cent of the total nitrogen soluble in 0.02 *N* sodium hydroxide, divided by per cent of total gossypol in the sample. This chemical index was found to be well correlated with results of chick feeding tests and with evaluations of available lysine in cottonseed meal samples (90).

Results of collaborative studies of cottonseed meal quality (64) support the value of the nitrogen solubility test as an index of quality. Progressive and more or less parallel decreases in nitrogen solubility and protein quality for the chick have been reported to result from increasing the autoclaving time of solvent-extracted cottonseed meal (91). Nitrogen solubility of cottonseed meal samples prepared under carefully controlled processing conditions has been reported to vary inversely with the energy input to the press during sample preparation (92). These samples, which showed varying degrees of nitrogen solubility, have been studied by other workers (93, 94), and their results, based on rat and chick feeding tests, show a general tendency for samples of low nitrogen solubility to be of low protein quality.

c. Tests Based on Reactions of Intact Protein

Heat treatment of foods, besides producing changes in solubility of the nitrogenous components, brings about other differences which involve proteins and which are detectable by chemical methods. The apparent adverse effect of heat processing of proteins on biological

availability of lysine is well known and has been discussed in detail elsewhere in this volume. (See Chapter 5.) Under conditions of heat and moisture, the free ϵ -amino group of the lysine of proteins undergoes reactions with reducing sugars and other compounds which affect the physiological availability of this amino acid. Thus, chemical methods of estimation of free amino groups of proteins might be useful for evaluation of protein quality. Harris and Mattill (95) observed that heat treatment decreased the number of reactive basic groups of intact proteins, as estimated by the Van Slyke method for amino nitrogen, or by formol titration. These heat-damaged products were found to have lowered susceptibility to hydrolysis by animal proteinases (95).

Almquist and Maurer (96) studied the relative quantities of reactive amino nitrogen in different samples of solvent-extracted soybean meal and showed that progressive increases in severity of heat treatment produced progressive apparent decreases in free basic groups in the meal proteins. A dye-binding method of estimation of basic groups (97) might be useful for estimating heat damage to protein quality, since, unlike the nitrous acid or formol titration procedures, varying solubility characteristics of the proteins of samples would not be expected to influence results.

A direct chemical method for estimation of "available lysine" in protein concentrates has been described by Carpenter and Ellinger (98a). (See also discussion that follows on pages 175–178.) The procedure is based on the observation that fluoro-2,4-dinitrobenzene reacts with free ϵ -amino lysine groups of intact proteins to form a lysine derivative which is stable in strong acid solution and may be estimated photometrically in the protein hydrolyzates. Presumably, ϵ -amino groups that had already been bound through reactions occurring during heat processing would not react with the test reagent and thus not appear as "available" lysine. Results of such chemical estimates of biologically available lysine in animal protein concentrates were reported to show highly significant correlation with results of chick feeding tests (98a). This method for estimation of the value of foods as sources of lysine has recently been applied to cottonseed meals (98b). (See also Chapter 17.)

d. Phthalein Dye Test

A method for the prediction of quality of soybean meal based on variations in ability of different meals to absorb or react with phthalein dyes has been described by Frölich (99). The quantity of dye removed by the sample from an acidic solution of cresol red is determined photometrically after an hour's contact of meal with dye. Heated meals ab-

sorb more cresol red than uncooked meals. Earlier work by Frölich (100) reported correlations between nutritive value, phthalein dye absorption, formol titration, and urease and antitryptic activities of soybean meals.

e. Fluorescence and Soybean Meal Quality

A fluorescence method for the detection of overheated soybean meals has been suggested (101, 102). The test involves fluorophotometric comparisons between phosphate buffer extracts of soybean meal samples and a standard solution of quinine sulfate. The quantity of soluble fluorescent materials in meals apparently is influenced by the severity of heat treatment. The method is reported to be an adequate means of detecting overheating in solvent-extracted meal, but of little value with screw-pressed products (86).

f. Other Chemical Indicators of Quality

There are tests for factors other than protein damage which are indicators of quality. Thus the tests for urease, antitrypsin, and hemagglutinins have a value in measuring quality of soybean meal; they are discussed in Chapters 5 and 14. Similarly, free and total gossypol are measures used extensively in evaluating cottonseed meal; they are discussed in Chapter 17.

4. Biological Availability and *in Vitro* Digestibility Tests

a. Biological Availability of Amino Acids; Influence on Biochemical Testing

In addition to the relative amounts of the various amino acids in a protein food, we must consider a number of other factors that influence the quantities and assortment of amino acids that are available for metabolic use.

(1) *Inherent characteristics of protein sources.* Examination of available figures for "apparent digestibility" shows that animal digestion of proteins, particularly those of plant origin, generally involves considerable "waste" of nitrogen (103). This may partly reflect the fact that plant tissues generally have less nitrogen per unit of dry matter, and excretion of "metabolic fecal nitrogen" appears to increase as the dry matter content of the diet increases (104). Because the use of protein materials in the animal feeding industry is so great, even small improvements in economy of utilization can be of significant practical importance.

As mentioned previously, the protein components of plants and ani-

mals are functional units of the living tissues from which they originate; hence it is not surprising that some proteins are found to have inherent characteristics, perhaps important in maintaining their integrity within the living cell, which protect them against degradative attack by proteolytic enzymes. For products of plant origin where the nitrogenous constituents are largely contained inside cellulosic supportive structures, mechanical factors may influence availability of amino acids by limitation of contact of the food proteins with the proteinases (105). Other factors affecting availability are presence of enzyme inhibitors and "damage" from processing.

(2) *Nature and state of animal.* It is difficult to restrict the scope of interest in amino acid availability to those characteristics of foods which influence their yields of amino acids to the animal. The digestive and absorptive capabilities of animals of different species are quite variable, and the diet itself may intensify this variation. Even within a given species, the maturity of development of the digestive tract may influence the efficiency of protein digestion. The human infant, for example, is unable to digest much of the protein of raw cow's milk (106), but the older child or adult digests raw milk proteins readily. Also, there is evidence to suggest that higher animals are able, when necessary, to effect adaptive changes in their digestive systems. Continued feeding of raw soybeans to chickens results in hypertrophy of the pancreas and marked increases in its content of proteolytic enzymes (107). Rats fed soybeans or peas show increased efficiency of absorption of dietary lysine as the feeding period is prolonged (67, 108). Such variations and instabilities of the digestive capacities of higher animals obviously increase the difficulty of development of appropriate *in vitro* biochemical tests for evaluation of amino acid availability.

(3) *Antibiotics.* Some factors that affect amino acid availability are at present impossible to evaluate by *in vitro* chemical means. For example, antibiotics commonly added to animal feeds probably affect the biochemical activities of the animal's intestinal microflora (109, 110). These changes sometimes improve the economy of utilization of dietary protein (46, 111). Even in monogastric animals such as the chicken, there is normally a fairly abundant microflora in the nutrient-absorptive portion of the intestinal tract. Antibiotic feeding has been shown to reduce the bacterial population of this part of the bowel (112), which in turn reduces the chances for chemical modification of amino acids by intestinal bacteria. Hence, antibiotic feeding tends to overcome some types of defects in availability of dietary amino acids (46, 47, 111). The effects of other nutrients are mentioned in a previous section.

(4) *Denaturation*. A number of proteins of animal origin are resistant to enzymatic digestion in their native or undenatured state. Egg albumin, for example, is resistant to attack by pancreatic trypsin (113) until it is denatured by heating or other physical or chemical treatment. Although egg albumin can be hydrolyzed by pepsin without preliminary heat denaturation, the optimum pH for its digestion is at a low value (114), and, since native albumin is readily denatured by exposure to acid, there is reason to believe that the rapidity of substrate attack may depend on the rate of denaturation or "unfolding" of the peptide chains of the albumin molecule in the acid digestion medium (115). Ovomucoid, a heat-labile protein fraction of egg white, is itself an active inhibitor of trypsin (116). Raw egg is slightly less well utilized by the rat, dog, and man than heat-denatured egg white proteins, but it appears nevertheless that most higher animals are able to overcome these difficulties in digesting the native proteins of eggs (117-120). Treatment with wet heat definitely increases the susceptibility of the proteins of whole cow's milk to *in vitro* attack by crystalline trypsin and chymotrypsin, but it is not clear whether this is the result of heat denaturation of the milk proteins or of some other effect of heating on the composition of the milk (121). Although evidence for improved digestibility of milk proteins by mild heat treatment has been observed with dogs (122) and with human infants (106), there is no clear-cut indication that heat denaturation is required for the efficient utilization of proteins of cow's milk by most animals. *In vitro* studies of the rate of tryptic attack of myosin showed no improvement to result from heat denaturation of this protein (113), and the undenatured proteins of muscle tissue are at least equal in digestibility and nutritional value for rats to the proteins of cooked meat (123-126).

Haurowitz and co-workers (113) concluded that denaturation is required for efficient attack of proteins such as egg albumin and serum globulins because the groups which the enzymes attack are relatively inaccessible. This comes about as a result of the positions of these groups inside the closely packed peptide chains of the native protein molecule. The observation that denaturation is not required for the tryptic hydrolysis of fibrous animal proteins such as myosin and fibrinogen is consistent with the concept that the peptide chains of such native proteins are already in a relatively expanded state.

(5) *Effect of protein structure*. Peculiarities of protein structure and of sequence of amino acids may affect biological availability of amino acids. It has been suggested (127) that the poor biological quality of arachin (a protein fraction of peanut) may be partly due to the arrangement of its component amino acids in certain enzyme-resistant

combinations, since disproportionately large amounts of the total lysine and histidine were found in that fraction of arachin that was resistant to *in vitro* hydrolysis. The elastin component of animal tissues is believed to owe at least part of its resistance to digestion to its high contents of proline and valine; these amino acids form peptide bonds that are relatively difficult to hydrolyze (128). There is some evidence that proteins relatively rich in cystine yield, on treatment with proteinases, "core" fractions which are resistant to further cleavage and which contain disproportionately large amounts of the cystine of the original material. This has been demonstrated with undenatured insulin (129) and wool keratin (130). The possibility that such an effect may be partially responsible for the impairment of absorption of cystine in rats fed raw soybeans (46, 131) has not been investigated.

b. In Vitro Digestibility Tests

Considerable progress has been made in the application of *in vitro* chemical and microbiological methods to the study of factors influencing digestibility. In spite of the availability of a number of relatively pure preparations of digestive enzymes of animal origin, it is not yet possible to simulate the complex mechanism for protein digestion that actually operates in the gastrointestinal tract of higher animals. In the animal, food proteins are exposed to multiple attack by a series of different proteinases and the products of hydrolysis are apparently absorbed promptly from the intestinal tract. During *in vitro* digestion, however, a complex mixture of end products accumulates as protein degradation proceeds. An adverse effect on the rate and ultimate extent of hydrolysis may be only one result of such an accumulation of hydrolysis products. Occurrence of coupled proteolytic reactions may cause qualitative changes in substrates that alter their susceptibility to enzymatic splitting (132).

The inability to duplicate precisely the animal's digestive processes is not altogether a disadvantage, however, for it may at times be desirable to isolate for study one part of the animal's digestive mechanism. The frequent choice of pancreatic digestion for such an isolated study is supported by evidence that pancreatic digestion is of considerable importance in the animal. Dogs deprived of pancreas secretions show serious impairment in ability to utilize protein (133); the amount of dietary protein assimilated by such animals corresponds fairly closely to that digested by the action of pepsin. Also, the feeding of crystalline soybean trypsin inhibitor to chickens has been shown to interfere seriously with protein utilization. This effect is largely reversed by addition of crystalline trypsin to the diet (134).

Most of the available *in vitro* methods for evaluation of protein digestibility have been developed for use with relatively narrow and defined classes of materials. As with the chemical methods mentioned previously, a method that is satisfactory for evaluation of the quality of soybean meals may or may not be useful for cottonseed meals. No attempt can be made here to discuss in detail all the attempted applications of *in vitro* digestion studies to problems of amino acid content and availability. But some of the published material that appears to be fairly representative of the techniques will be mentioned in the hope that this may be helpful in defining the problem and in suggesting further work.

TABLE II^a
RATE OF HYDROLYSIS OF PROTEINS BY PANCREATIC ENZYMES
AS AFFECTED BY HEAT TREATMENT

Sample	Type of processing	Extent of hydrolysis of proteins		
		After 1 day	After 2 days	After 5 days
		%	%	%
Whole egg	Raw	1	8	12
	Hard-boiled	11	16	31
Milk	None	19	24	28
	Skimmed and spray-dried	29	33	35
Whole oats	None	19	26	30
	Autoclaved, 30 min. at 15 lb.	17	22	27
Defatted wheat germ	None	29	38	52
	Autoclaved, 30 min. at 15 lb.	16	29	38
Beef blood	None	4	8	16
	Proteins heat-coagulated	31	47	60

^a According to D. Melnick and B. L. Oser, *Food Technol.* **3**, 57 (1949).

Melnick and co-workers (135, 136) have stressed the importance of rate of enzymatic hydrolysis of dietary protein in determining the physiological yield of amino acids to higher animals. They have described (136) an *in vitro* method designed to study differences in rate of enzyme attack of the proteins which involves treatment of a solution or suspension of the food with a mixture of pancreatic enzymes under controlled conditions of pH and temperature. After 1, 2, and 5 days of digestion, the degree of hydrolysis of the proteins of the sample is estimated by a formol titration procedure. Their results (see Table II) indicate that this method is capable of detecting differences, resulting from heating, in the susceptibility of proteins to enzyme digestion.

The above-indicated increases in rates of protein hydrolysis associated with moderate heat treatment of egg, milk, and blood proteins are in agreement with the findings of others (113, 121); however, as discussed earlier, such indications of improved pancreatic enzymolysis are not necessarily reflected in

feeding tests with higher animals. But if the application of heat to a product is severe enough to depress measurably the *in vitro* hydrolysis of its proteins by pancreatic enzymes, this change is often actually detectable by feeding tests.

An *in vitro* method involving digestion with crystalline pancreatic trypsin has been found useful for evaluation of protein quality of roller-dried whey (137). Twenty-four-hour digests were prepared; after removal of undigested proteins, lysine was determined microbiologically and formol titrations were carried out. An approximately linear relationship was found to exist between the quantity of lysine released by trypsin and the biological value for rats of the various roller-dried whey samples. Lysine liberation after successive digestions with pepsin and a trypsin-chymotrypsin mixture was reported to be less reliable as a means of prediction of quality than was digestion with trypsin alone.

Evans and co-workers (138) have applied both *in vitro* enzyme digestion tests and *in vivo* tests with growing chickens to the problem of determining the effects of heat treatment on protein quality of soybeans. They observed positive correlation between protein efficiency for the chick and the susceptibility of the sample protein to *in vitro* hydrolysis with trypsin and erepsin. When digestion with trypsin and erepsin was preceded by treatment with pepsin, however, the relatively poor digestibility of the raw meal was no longer apparent. Similar findings have been reported by Liener and Fevold (139).

In vitro digestion tests have been used in attempts to evaluate the protein quality of cottonseed meal. Ingram and co-workers (140) digested various cottonseed meal samples with pancreatic enzymes and studied amino acid liberation by microbiological assay. They found good correlation between release of microbiologically available lysine and sample quality as indicated by chick growth tests. Similarly, other workers (93) reported generally good agreement between results of rat feeding tests and pancreatic release of amino acids from cottonseed meal as measured by a growth test with *L. mesenteroides* P-60.

Some of the difficulties of interpreting the results of *in vitro* digestion studies have been pointed out by Denton and Elvehjem (141). They studied the rates of liberation of amino acids from beef muscle, casein, and zein during *in vitro* digestion with animal proteinases. Although significantly different patterns were found in the rates of release, they conclude that it is difficult to designate one pattern as favorable and another as unfavorable because of lack of knowledge of the pattern of release of amino acids best suited for their efficient utilization by higher animals.

A rather complex *in vitro* method for prediction of the biological value of protein foods has been described by Sheffner and co-workers (142). Here the criteria are the differences observed among various protein sources in their total contents of amino acids and in the patterns of amino acids made available by *in vitro* digestion with pepsin. The authors stress their belief that differences among proteins in susceptibility to proteolytic attack by pepsin and the characteristics of the end products of peptic hydrolysis may reflect variation in over-all protein quality. Although this may be true, direct evidence is not available. This method minimizes the importance of the most deficient amino acid as a determining factor for quality in a manner similar

to the earlier integration methods (73, 74). Also, this method obviously depends heavily on the amounts and distributions of the amino acids made available to microorganisms by treatment with pepsin. Yet many food materials, being of animal or plant tissue origin, are known to contain significant amounts of other proteolytic enzymes; no attempt is made to distinguish the hydrolytic effects of added pepsin from those of naturally present proteinases.

5. Performance Tests with Microorganisms

Some interesting studies have been made of the possibilities of use of ciliated protozoa of the genus *Tetrahymena* in studies of the nutritional quality of proteins. These organisms are known to possess proteolytic enzymes (143, 144) and are able to utilize intact proteins as sources of amino acids (145). The results of studies of the nitrogen metabolism of *Tetrahymena geleii* H have been summarized and discussed by Kidder and Dewey (146) and by Johnson (147). It is evident that the amino acid requirements of this organism resemble, in many respects, those of higher animals. The resemblance in requirements, the rapid growth and small size, together with the ability of *Tetrahymena* to hydrolyze proteins, suggest that they would be useful in protein evaluation.

Casein, lactalbumin, and gelatin were tested by Dunn and Rockland (148) as sources of dietary nitrogen for *T. geleii* H. The protozoa were grown in sterilized media lacking all amino acids, but adequate in other nutrients. The protein materials were dissolved or suspended in the culture medium at concentrations up to approximately 1.0 mg./ml. Growth was estimated by titration of the acid produced, and relative "biological values" were calculated, using an assigned value of 100 for casein. Results of this test showed general agreement with results of feeding tests with higher animals. It was pointed out, however, that the quality ratings of proteins varied with the level of protein added to the culture tube and with the length of time of incubation.

In an improved method of evaluation (149), an attempt was made to take these factors into account. It was found that a long incubation period in the *T. geleii* tests gave results in best agreement with those obtained by the nitrogen balance methods with rats.

Anderson and Williams (150) have called attention to the fact that *T. geleii* are essentially aerobic organisms and that, since the quantity of acid produced is dependent on the concentration of oxygen in the culture medium during the test period, the validity of acid production as the criterion for growth is questionable. A practical disadvantage of this method of following growth is the necessity for a 40-day incubation period. Anderson and Williams (150) employed a method of relating the amount of growth of the test organism to enzymatic activity contained in the cell suspensions. This procedure consists in spectrophotometric measurement of the red triphenylformazan produced by enzymatic reduction of 2,3,5-triphenyltetrazolium chloride. Pilcher and Williams (151) used this method for investigating protein quality. Growth in cultures was estimated by the spectrophotometric method after 3, 4, or 5 days of incubation of assay tubes, and casein was the standard for quality

comparisons. The authors conclude that quality values calculated on the basis of results of the 3-day test agree closely with those obtained by rat feeding tests, and that their agreement is better than those from cultures incubated for 5 days.

In the studies with *Tetrahymena*, the protein samples to be tested were subjected to considerable heat treatment during sterilization of the assay tubes. Viswanatha and Liener (152) have investigated the effects of such heat treatment on the utilization of various proteins by *Tetrahymena geleii* W. Their results indicate that wet heat treatment sufficient for protein denaturation greatly improved utilization of the proteins of a crude extract of raw soybeans, of a preparation of soybean globulin, and of egg and serum albumins. Since such improvement in response to the proteins of the crude soybean extract could have resulted from heat destruction of trypsin inhibitor or toxic hemagglutinin components of soy, the authors tested the effects of addition of purified preparations of these substances on utilization of casein by the organism. No evidence of inhibition of growth of the protozoan by soy trypsin inhibitor or hemagglutinin was seen. The authors conclude that the poor growth responses of the organism to the unheated soybean proteins or the undenatured albumins probably represents an inability of the protozoan to utilize these proteins in the native state. Partially confirmatory evidence for this was obtained by *in vitro* testing of the proteolytic activity of a proteinase isolated from a growing culture of *Tetrahymena*. It was found that denaturation of substrate by heat or urea greatly increased the rate of attack. This was true for hemoglobin, soy globulin, and β -lactoglobulin; fibrinogen and casein were readily hydrolyzed even without heat treatment. As pointed out by the authors, ability of a proteinase to attack undenatured fibrinogen or casein and inability to hydrolyze native albumins or globulins is also noted with "trypsins" of pancreatic origin.

Although the results of Viswanatha and Liener with *T. geleii* indicate that protein utilization by this organism is influenced by some of the same factors believed to affect utilization in higher animals, several differences in behavior of *T. geleii* and larger animals are evident. The effectiveness of methionine supplementation of raw soybean proteins in improving protein utilization by rats and chicks (153, 154) could not be demonstrated with *T. geleii*. This may of course be due to abnormally low requirements by this protozoan for sulfur amino acids; however, no obvious indication that the requirements of sulfur amino acid for cell formation by this organism are unusually low is evident in the results of Kidder and Dewey (146) or from the data on amino acid composition of *T. geleii* cells (151). Another finding of Viswanatha and

Liener that appears to cast some doubt on the general validity of predictions of protein quality for higher animals on the basis of growth tests with *T. gelei* is that neither β -lactoglobulin nor hemoglobin would support growth of this protozoan as the source of protein in the medium.

Rosen and Fernell (155) have used *Tetrahymena pyriformis* W, which is also called *T. gelei* W, to study twenty different vegetable and animal foodstuffs, without prior separation of the protein. They compared their results with rat assay data obtained by other investigators for similar products and found general agreement. The authors have shown clearly that the composition of the medium, especially that of its glucose content, is very important in protein evaluation. Furthermore, criteria in addition to growth (such as ammonia-nitrogen production) are necessary for successful use of the method (156).

In the *T. gelei* studies, the test protein serves as the sole source of amino acids available for growth of the organism. Since this protozoan is able to hydrolyze intact proteins, the preparation of the sample for testing does not require a separate hydrolysis step. Other methods of microbiological evaluation of protein quality have been proposed which are similar to the *T. gelei* tests in that the test protein must furnish all the amino acids required for growth of the organism, but differ in that the proteins are hydrolyzed by some method independent of any activity of the test organism.

In the procedure of Halevy and Grossowicz (157) the materials to be investigated are digested *in vitro* for 48 hours with a crude preparation of pancreatic proteinases. The resulting hydrolyzates are analyzed for free amino nitrogen by the Van Slyke nitrous acid method and, after suitable dilution based on amino nitrogen content, tested as sources of nitrogen for growth of *Streptococcus faecalis*. The quantity of each hydrolyzate that must be added to a culture tube in order to obtain approximately half-maximum growth of the test organism is determined by photometric measurement of culture turbidities after incubation for 48 hours.

The hydrolyzates of the test proteins were further tested to determine which of the ten amino acids believed to be essential for the organism was actually responsible for the observed limitation of growth. This information was obtained by supplementation of the basal medium with nine of the ten essential acids and retesting of the hydrolyzate with the supplemented medium. The amino acid which, on omission from the basal medium, failed to allow an increased growth response to the hydrolyzate was chosen as the one most deficient.

A method of evaluation similar, in some respects, to that of Halevy and Grossowicz has been applied to evaluation of cottonseed meal quality (93). *Leuconostoc mesenteroides* P-60 was used for hydrolyzate evaluation. This organism is known to require the simultaneous presence of fifteen different amino acids for growth, including all those known to be indispensable for the growing rat or chick (158). *In vitro* enzyme digestions of the cottonseed meal

samples were carried out by successive treatments with pepsin, trypsin, and hog mucosa for 24-hour periods. The samples were also hydrolyzed with acid in order to obtain, for comparative purposes, hydrolyzates representing complete liberation of the amino acids actually present in the sample. Hydrolyzates, both acid and enzyme, were added to assay tubes at graded levels, and growth of the test organism was estimated, after 3 days of incubation, by measurements of acid production. "Indexes of protein value" were computed from the titration data in such a way as to represent the relative abilities of the enzyme digests to serve as sources of amino acids available for growth of *L. mesenteroides* P-60. Theoretically, such values should be influenced by a number of different factors, including the quantitative requirements of the test organism for amino acids, the relative amounts of these actually present in the protein source, and the susceptibility of the proteins of the source to enzyme digestion under the conditions chosen for testing. The results given by the authors indicate reasonably good agreement between values obtained by this microbiological method and those determined by rat growth.

In the above procedures with protozoa or bacteria as test organisms, the protein or protein hydrolyzate is tested as the sole source of amino acids available for growth. Although this practice has the advantage of being less time-consuming than the systematic quantitative assaying of materials for their contents of individual amino acids, it has several obvious disadvantages. Unless supplemented by additional information, the results give no indication of the cause for failure of a protein source to allow good growth of the test organism. Thus such methods are also subject to the same criticism that has been applied to the determination of biological value with higher animals, i.e., that the situation is analogous to attempting to determine the adequacy of a given food with respect to all the different vitamins by means of a single growth experiment (159).

The reliability of such a microbiological method of evaluation as a basis for prediction of quality for higher animals obviously depends heavily on the existence of a high degree of similarity in proportions of the various amino acids required by the microorganism employed and by the higher animal. But some of the results available suggest that there may be important differences. As pointed out by Viswanatha and Liener (152), no improvement in growth of *T. geleii* resulted from methionine supplementation of soybean protein; this is different from results of rat and chick studies. Tryptophan is absent from gelatin (70), but it is not clear from the data of Halevy and Grossowicz (157) whether the observed growth response of the test organism to a gelatin hydrolyzate was due to use of a strain of *S. faecalis* lacking an absolute requirement for tryptophan or resulted from the tryptophan contributed by the enzyme preparation used for digestion. In either case, the information that gelatin supports only 29% as much growth of *S. faecalis*

as does casein seems to be of less general nutritional usefulness than the knowledge that gelatin is devoid of tryptophan. Moreover, the results of Halevy and Grossowicz with *S. faecalis* indicate that lysine is the first limiting amino acid in egg albumin and that serious deficiencies in arginine and histidine also exist. But results with rats show no improvement of albumin by lysine addition and indicate the first limiting acid to be methionine (75). Similarly, with casein, the *S. faecalis* tests indicated this protein to be deficient in lysine, arginine, isoleucine, and threonine, whereas for the rat the most important deficiency of casein appears to be in content of the sulfur amino acids (160); for birds arginine is the most important deficiency (161).

IV. CONCLUSIONS AND TRENDS

Nutritionists now have many different techniques for evaluating protein foods. Some of these are more useful than others because of sensitivity, or of accuracy, or of simplicity. No one method is complete in itself, because no single method can express all the uses to which the results will be put.

The great need in this field is for methods that yield measures of the amounts of each of the amino acids that is available to the animal. We must soon stop employing terms such as protein value, protein availability, and protein digestibility, and find instead relatively simple terms for expressing amounts of individual amino acids that are available to a particular animal. Ideally these methods should be chemical ones, but until these can be devised, bioassay techniques will be needed. These are at present limited in range, and not nearly good enough to meet our needs.

The problems of protein evaluation are, of course, not limited to vegetable materials. Indeed, the problems posed by animal materials may be even more knotty. All, however, must eventually yield to new approaches of evaluation.

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CHAPTER 8

USE OF PROCESSED PLANT PROTEINS IN MIXED FEEDS

N. R. ELLIS

I. INTRODUCTION

It is the purpose of this chapter to present the principles underlying the use of processed plant proteins in mixed feeds. References are given to standard sources of information on feed formulas, and several selected diets are presented merely as illustrative of the principles. The treatment, therefore, is general; specific applications will be found throughout the book, particularly in the chapters of Part II, where the use of specific processed plant proteins in mixed feeds is discussed. Also, in a number of chapters in Part I there is reference to the considerations which govern the selection and use of various plant sources of nutrients.

This discussion deals with practice in the United States; it is expected that practices elsewhere differ only in detail, depending on local sources of nutrients and economics of animal production.

* * * * *

Long before scientific knowledge established the essentiality of proteins in mixed feeds, livestock men were using processed plant products to augment their feed supplies. Usually this practice can be traced to the availability of these products in areas adjacent to milling plants, their relative cheapness, and the shortage of other common feeds, especially the cereals. Early in the century, before soybeans were grown extensively in the United States, importations from the Orient led to the use of soybean meal as a feeding-stuff on the West Coast.

Early use of cottonseed and peanuts as feed in the South was in the form of the seed. The introduction of oil mills, with production of cottonseed meal as a by-product of oil manufacture, led to use of the meal as a feed. Because of the low volume of corn production, cottonseed meal formed a major concentrate feed for cattle for many years. In fact, this practice persisted almost up to World War II, partly from tradition and partly because of low costs in comparison to cereal grains

and other carbohydrate-rich feeds. Other plant protein feeds likewise were used without much regard to balancing the proportion of protein to carbohydrates in livestock rations. Peanut meal followed peanut as a feed for hogs. Even in the 1920's it was common practice to feed to fattening hogs as much as one-third of the ration as peanut meal, although this was easily twice as much as needed to supply the required level of protein. Availability and cheapness were prime considerations.

In the North, especially the Midwest, linseed meal along with cereal by-product feeds played much the same role as did cottonseed and peanut meals in the South, although general abundance of corn and other cereal grains limited their use. Livestock feeders generally recognized certain values in the oilseed and other plant protein concentrates even before appreciation of the protein values became general as the result of scientific data developed by the Agricultural Experiment Stations. For instance, linseed meal was valued in the ration for its conditioning properties and the sleekness of the hair which resulted. The laxative property of wheat bran is another example. Wheat middlings came to be a popular pig feed at an early date partly because they made a fine-textured slop when mixed with other feeds. When slop was replaced by the self-feeding of dry materials, this popularity declined.

With the advent of commercial mixed feeds, the development of scientific data on the nutritional properties of specific feeds, and general adjustment of relative prices, most of these older practices have been replaced. Today, processed plant proteins are included in rations and diet formulas to meet specific nutrient requirements. Instead of a single protein-rich product to supplement corn, for example, most diets contain several. To some extent, the pendulum has swung back to soybean meal in swine and poultry diets. This has been done, however, with full recognition of the nutrients required to make a complete and balanced diet.

World War II brought about an awakening to the relatively short supply of protein feeds. Production in the United States has been insufficient to meet the needs of livestock from a nutritional standpoint. The commercial production of mixed feeds, especially for poultry and to a lesser extent for swine and cattle, has produced demands for more protein feeds. Decreased importations of copra and soybeans from the Orient have been met in part by importations from other parts of the world of a variety of other seeds and meal products. Nevertheless, the bulk of the plant protein concentrates are made from a few products grown in the United States. The list is headed by soybean meal and

includes cottonseed, linseed, and peanut meals along with alfalfa meals, and milling, brewery and distillery by-products.

II. VEGETABLE PROTEINS

Why are vegetable protein concentrates included in mixed feeds, and how are they used? First and foremost is protein. Most of the processed plant products discussed in this volume are relatively rich in protein. With few exceptions they contain more protein than the cereal grains which generally constitute the basal components of diets. The oilseed meals as a class are the highest in protein content; the milling feeds, with some exceptions, are the lowest. The quality of protein is perhaps more important than quantity, at least for swine and poultry. Particularly important is the property of a supplement or combination of supplements to provide high enough levels of those essential amino acids that are deficient in the basal feed, generally corn, to bring the mixture up to requirement levels for the species and its stage of development. Sometimes this is not completely feasible economically, and one or more amino acids in shortest supply may be added in highly concentrated form to complete the diet. (See Chapter 13.) With information available on the requirements for protein and for the essential amino acids, it is unusual for diets to be formulated that are excessively high or far above the requirements of the animal to be fed. Usually price considerations are a deterrent to abnormal usage of protein feeds, whereas this was not so true in earlier years.

Processing methods for products that provide for maximum digestion and utilization of the protein are highly important in promoting the use of a given class of concentrate.

Some importance is placed on the mineral contributions of plant protein concentrates. Except for the legume forage meals, such as alfalfa, calcium contents are low. Phosphorus, however, may be high enough to correct the low and sometimes deficient levels in cereals and forages. Considerable variation exists in the trace elements. Furthermore, the data on such elements as cobalt, zinc, iodine, and copper are rather fragmentary; accordingly, little use has been made of specific feeds to build up levels of trace minerals. Copra meal, however, appears to have an exceptionally high cobalt content. Whether some of the value of linseed meal, especially as a cattle feed, is traceable to its moderately high cobalt content is not known.

Vitamin contributions to diets are largely among the water-soluble or B factors rather than the fat-soluble group. Exceptions are alfalfa meal and other green leaf products, especially if dehydrated, which are high in carotene content and are often included expressly to provide

the animal a source of vitamin A. As a group, the plant protein products are not outstanding as sources of thiamine or riboflavin. A few, such as peanut and safflower meals, are sufficiently high in pantothenic acid to be worthy of attention. Peanut meal is also outstanding as a source of niacin. There are a few special fermentation products that are high in some of these vitamin factors, such as riboflavin, and thus fill specific functions in diets.

Above all, the plant protein concentrates, like other feedstuffs, must meet other qualifications, particularly palatability and freedom from injurious ingredients. There are variations in palatability and, hence, there are limits in some cases to the amount that can be incorporated in diets, depending largely on the class of animal. Certain properties, such as laxativeness, need to be recognized and levels in the diet adjusted accordingly. More often than not, a product will be added because of its mild or moderate laxative properties. Relative bulkiness and fiber content are among other considerations.

In formulating diets, therefore, a truly scientific approach should involve considerations of palatability, protein content and quality, and supplementary relations with the basal feeds. Other considerations are the amino acids, vitamin and mineral contributions, digestibility, compatibility in mixing and pelleting, and general over-all suitability for feeding to the class of animal. Nutrient requirement data and types of formulas are available for guidance, including publications of the National Research Council (1) and numerous other sources (2-5). (See also Tables II and III, Chapter 2.) The National Research Council publications are essentially compilations, summaries and adaptations of feeding and nutrition experiments that express the known requirement values for the required nutrients. Availability and cost factors must be major considerations in governing the choices and amounts of feed products in diets. Special mineral, vitamin, amino acid, and other products may need to be added where it is not feasible to depend on the main group of feedstuffs.

III. RUMINANTS VERSUS NON-RUMINANTS

Each class of animal has characteristic diet and ration needs. Likewise, stage of development and purpose for feeding dictate different diets: whether for growth from the newborn to the mature stages, or for fattening, maintenance, or reproduction. Ruminants, including cattle, sheep, and goats, have feed requirements that are characteristically different from those of non-ruminants, such as poultry and swine. The digestive system of ruminants, with its compartmentalized set of four stomachs, permits these animals to utilize forages and other coarse

roughages. The microflora of the rumen aid in breaking down these bulky and coarse feeds; cellulose, particularly, is utilized in this manner. The microflora, as they grow and multiply, synthesize cellular constituents from the breakdown products. Among these are various vitamins of the B or water-soluble group. It is because of this fact that ruminants are not dependent on feed sources for these vitamins. Until rumen function is established in the young calf or lamb, however, there is need for this vitamin supply as well as other nutrients ordinarily produced by microorganism synthesis.

Another important difference between ruminants and non-ruminants is the requirement for essential amino acids and high-quality protein. Just as vitamins are synthesized in the rumen, so are amino acids and proteins. It is presumed that as the microflora die their cells break down and the released proteins are utilized by the ruminant animal. Through this process, non-protein nitrogen compounds such as urea when included in the diet are utilized in the building up of amino acids and proteins. (See also Chapter 12.) The proteins in the feed supply go through a breaking-down and rebuilding process. In the case of the unbalanced or poor proteins that are deficient in amino acids which are essential for growth and not normally synthesized by the tissues of the host animal, amino acids synthesized by the microflora are presumably used in the rebuilding of the proteins going into the muscle structure of the animal. Many studies have been carried out in recent years on rumen function and the factors involved in optimum feeding of ruminants. Much importance has been placed on the optimum nutrition of the microflora as the primary prerequisite to the nutrition of the host animal. This by no means implies that the entire nitrogen intake of the ruminant can be in non-protein form, or that it is desirable that no B vitamins be included in the diet. Nevertheless, it has generally been difficult to show any real differences between protein concentrates when protein quality was the only apparent variable.

Because cattle, sheep, and goats subsist basically on forages, first consideration for supplementation with processed plant protein concentrates comes about when the forage is inadequate for the production purpose at hand. In the case of animals on the range, this may be a fall and winter deficiency in protein content of the forage plus perhaps a deficiency of phosphorus and vitamin A and even energy. Formerly, the prevailing practices were simply to feed either an oilseed meal product, such as cottonseed, or hay, preferably alfalfa. With the recognition of the possible multiple deficiencies, it has become common practice to feed a mixed supplement that contains two or more sources of nitrogen; other supplements that supply minerals, especially phosphorus;

a vitamin A or carotene source; and some carbohydrate-rich feeds, generally cereal grains with perhaps some molasses. The protein (or protein equivalent) content may be approximately 20% instead of 40%.

A wide range of choices of plant protein products is possible for supplying the protein or nitrogen requirements along with part or all of the phosphorus and energy needs. The choice is largely a question of availability and costs. Alfalfa meal is commonly incorporated in the mixture to supply carotene; moreover, mineral elements in alfalfa have been found highly beneficial in the nutrition of the rumen microflora. Because these present-day supplements are intended to serve a broader purpose than the single protein concentrates of earlier practice, the quantity fed is ordinarily larger. Thus, about 2 pounds is needed to supply the equivalent in protein supplied by 1 pound of the oilseed meal. Such a supplement mixture is essentially equally effective in wintering the breeding herd or steers and herd replacement stock. Much the same type of supplement is given to range sheep. Here, however, more dependence is placed on alfalfa hay with perhaps some grain supplement if the sheep flock is reasonably near an irrigated farming district.

Plant protein concentrates are employed extensively in farm and feed-lot production of beef cattle and sheep, especially in fattening rations. Increasing use is being made of mixed supplements. Formula feeds for farm cattle herds are likely to contain a wide variety of concentrate products with costs largely influencing the choice in a given year within the limits of the need to maintain a standardized product as to nutritional adequacy, palatability, and other feeding and handling properties. In the fattening of steers, the daily ration may be a relatively simple one. For example, it may consist of 10 to 20 pounds of corn, depending on the size and stage of fattening, along with 2 pounds of an oilmeal, with a choice of soybean, cottonseed, linseed, or peanut meal, about 5 pounds of mixed hay, and corn silage from 15 pounds to 40 pounds according to appetite.

A somewhat more complex concentrate mixture for winter feeding of young stock, where a moderate rate of gain is desired below that required for rapid finishing for market, is as follows:

Cottonseed meal	300 pounds
Soybean meal	300 pounds
Ground yellow corn	400 pounds
Steamed bone meal	10 pounds
Salt with traces of cobalt, iodine, and iron	10 pounds

Such a mixture can be fed with medium-grade hay or grass silage. When the forage is likely to be low in carotene, a vitamin A supplement should be added. Two to three pounds of the mixture should supply adequate amounts of protein to supplement the low-protein forage fed to appetite. In addition, the protein concentrates, the corn, and the bone meal supply phosphorus; and the salt and the traces of cobalt, iodine, and iron add other essential elements.

Dairy cattle consume large tonnages of the protein concentrate feeds, mainly as supplements to grains in the feeding of milking cows. The staple feeds are the milling by-products, the oilseed meals, and the brewery and distillery by-products. From the standpoint of nutrient balance, chief emphasis is on protein supply although phosphorus supplementation is important as is over-all palatability of the mixture in the feeding of high-producing milk cows.

The concentrate mixtures are usually grouped according to protein contents, such as 12, 16, or 18%, and the mixture or protein content is based on the supplementary needs of the roughage being fed. Thus, if it is a low-protein roughage, a higher protein mixture is selected; if the roughage has a high protein content, as for example alfalfa hay, a lower protein content in the concentrate mixture will suffice. Typical concentrate mixtures may simply consist of corn, oats, and an oilseed meal in proportions to provide the desired protein level. Frequently milling products are included.

The plant protein concentrates come into use in the feeding of sheep on the farm and in the feed lot when the forage is relatively low in protein or when it is not highly palatable. Thus, breeding ewes may be fed daily a quarter of a pound of an oilseed meal and three-quarters of a pound of shelled corn when the forage consists of a combination of oat straw and corn silage. In the fattening of lambs, one part of a protein concentrate such as cottonseed meal, linseed meal, soybean meal, or distillers' grains and seven parts of corn is commonly used. Special and more complex mixtures prepared in feed mixing plants are also given.

IV. NON-RUMINANTS

Non-ruminants require more care than ruminants in the selection of feeds and in the balancing of nutrients to meet production requirements. Pastures and ranges which form the basic feed source of ruminants provide more of an all-purpose feed than do any of the concentrate feeds on which swine and poultry depend. Furthermore, non-ruminants depend on feed sources for more of the nutrients. This includes amino acids, essential for the particular species, which in turn places

emphasis on protein quality. In addition there are the B vitamin factors such as riboflavin, pantothenic acid, niacin, and vitamin B₁₂ which are all too frequently not in great excess and often below requirements in the average concentrate feed mixture.

Great stress was formerly placed on animal proteins in the formulation of the more efficient swine and poultry diets. With the unraveling of the animal protein factor plus other new information on nutritional requirements and new and improved feed supplements, especially the plant protein products, this dependence on animal proteins no longer governs diet formulation. At the same time the great increase in soybean production provided a meal that was suitable as the major protein supplement. Nevertheless, the animal protein products continue to have an important place. Current practices show a trend toward fewer protein concentrates in the diet mixtures than was true during the 1940's. Special supplements in the form of mineral and vitamin products have increased, no doubt.

1. Swine

In swine production in the Corn Belt, corn forms the basal feed. The supplemental feeds, usually as mixtures, are built around the requirements for feeding of the pregnant and lactating sow, for the early development of the young pig, then for the intermediate or growing stage, and finally for the fattening stage. Protein requirements vary with the stage of the life cycle, the highest being for the young pig and the lowest for the fattening shoat. Energy content is generally adjusted also, with the pregnant sow receiving the lower energy, more bulky mixtures, and the young pig the higher energy, more concentrated types of diets. These considerations help to govern the choice and proportions of plant protein concentrates in the mixtures intended for the several feeding stages. Within limits, the protein components of the diets may remain the same in the ration but the proportions adjusted in the entire diet. Thus the protein supplements may be as simple as soybean meal alone, or two parts of soybean meal and one part of tankage, or more complex mixtures such as equal parts of soybean meal, tankage, cottonseed meal, linseed meal, and alfalfa meal.

The complete diet of the growing pig from weaning to 100 pounds in weight may consist of approximately 70% of ground corn, the rest consisting of a protein mixture along with a mineral mixture, usually not more than 2% of the total, and lesser amounts of antibiotic and vitamin supplements. The composition of such a supplement is illustrated as follows:

	%
Soybean meal	30
Linseed meal	15
Wheat standard middlings	20.4
Tankage, digester	15.5
Alfalfa meal, dehydrated	15
Ground limestone	1.7
Steamed bone meal	0.7
Salt, plus traces of copper, zinc, manganese, and iron	1.7
Chlortetracycline, 20 mg./lb.	
Vitamin B ₁₂ , 20 γ /lb.	
Vitamin D, 90 I.U./lb.	

When 30 parts by weight of this supplement is used with 70 parts of corn, the combination will contain approximately 16.25% protein, 0.67% calcium, and 0.52% phosphorus, a composition which is considered adequate for feeding pigs weighing about 50 pounds. The copper, zinc, manganese, and iodine additions, incorporated with the salt, may be of the order of 6, 65, 40, and 0.25 mg., respectively, of these elements per pound of the supplement mixture so as to provide approximately 2, 20, 12, and 0.07 mg., respectively, per pound of complete diet. Likewise, the amount of antibiotic supplied (chlortetracycline) will provide about 6 mg./lb., and of vitamin B₁₂, about 6 γ /lb. of complete diet. From 100 pounds to market weight, downward adjustment of protein content is ordinarily made at least once and sometimes twice until the corn or grain combination constitutes at least 80% and possibly as much as 90% of the entire diet.

2. Poultry

Research in poultry feeding and nutrition has produced a greater fund of information on nutritional requirements and diet formulation than for any other class of livestock. It has resulted in pronounced increases in efficiency of feeding, especially in the growth of chickens and turkeys. Most of the feed for broilers and poults is commercially mixed. In general, these mixed feeds are compounded to meet exacting standards, and ingredients are selected with great care. The multiplicity of nutrients that need to be considered in compounding poultry diets is well illustrated in the National Research Council report of 1954 on nutrient requirements for poultry (1). Thus, for starting chicks there are specific requirements for six mineral elements, ten vitamin factors, and eleven amino acids, and tentative standards for several more of these nutrients. (For older chickens, requirements for some of the nutrients are not well established.) Furthermore there is

a great fund of information on the general feeding values and combining properties of a great number of individual feeds. Recently, attention has been focused on the development and use of high-energy diets for growing poultry. This has called for a reappraisal of the requirements for individual nutrients. Thus the selection and use of feeds in formulating diets has become a complex scientific procedure. (See Tables III and IV in Chapter 13 for examples of diets for broilers.)

Of the plant protein concentrates discussed in this volume, many of them can be and are included in poultry diets. The chief ones are the oilseed meals, the milling and fermentation by-products, alfalfa, and related meals. Soybean meal has become the one of first choice. Improvements in manufacture, especially aimed at proper heat treatment to bring out the best in nutritive value of protein, has made this meal more or less a standard of reference. Values of other protein concentrates are commonly compared on the basis of substitution of various percentages of soybean meal. On this basis, peanut meal is considered a satisfactory replacement for a part and sometimes all, especially if animal protein is also used in the diet. Processing of cottonseed meal as it affects gossypol removal and nutritive value of the protein influences the replacement value of this meal. Cottonseed meals are being produced, however, which can effectively replace up to half the soybean meal. Linseed meal has been found to have a growth-depressing effect in poultry diets which has not been fully overcome by processing changes. Sesame meal, available in limited quantities in the United States, has proved quite satisfactory. Of the other less common oilseed meals, poppyseed has been found satisfactory at 5% and 10% levels. Palm kernel meal is also satisfactory in combination with soybean meal and fish meal. Sunflower seed meal shows moderately good substitution value. Mustard seed meal appears inferior to sunflower seed meal, and rapeseed meal inferior to mustard seed meal. Tung oil meal, because of its low palatability and toxic properties, is rated as unsatisfactory.

Dried yeasts, of the wood or torula type, serve as satisfactory supplements for part of the protein and also as sources of B vitamins. Corn and sorghum gluten meals are frequently included along with soybean meal. Although wheat middlings products do not have the high protein content of most of the other plant protein meals, they find frequent use. Distillers' solubles from various grain sources are also valuable at moderate levels. Alfalfa leaf meal and dehydrated alfalfa meal are commonly added as a source of carotene; the level of these meals ordinarily ranges from 2 to 5%, the lower amounts being favored in high-energy diets, and hence their protein contribution is rather negligible.

In the selection of feeds for a broiler diet, for example, the adequacy

of the supply and the current price are necessarily prime factors. Ordinarily, ground yellow corn is the basal feed, with sorghum grain a partial replacement in areas close to supplies of this feed. Soybean meal as mentioned previously, has come to be preferred for the first protein concentrate to be added. A choice is next made of other plant protein concentrates including peanut meal, corn gluten meal, a properly processed cottonseed meal, or others of the group just discussed. Then moderate amounts of fish meal and meat scrap are also generally added to help meet needs for essential amino acids plus other nutrient factors. In deciding on the relative percentages, a careful and logical approach gives due weight to the required total percentage of protein in the diet and the best practical balance of essential amino acids that comes nearest to meeting the requirement standards based on the National Research Council's published figures (1).

In completing the diet, of course, there are vitamin and mineral supplements to be added, the chief ones being riboflavin, vitamin A, vitamin D, vitamin B₁₂, salt, calcium, phosphorus, manganese, and perhaps other trace minerals. It is also customary to add an antibiotic supplement.

In some of the starting and growing mashes, as well as in diets for the breeding flock, ground wheat, ground oats, wheat middlings, and wheat bran may be substituted for a part of the corn and some of the other plant protein supplements mentioned for broiler diets. Alfalfa leaf meal is also generally included.

Diets for laying hens may be combinations of mash and grain mixtures. In the case of the breeding flock more care needs to be exercised in meeting the nutrient requirements than for hens kept only for egg production. Partly for this reason, all-mash diets are preferred because they give more uniform results. A typical diet showing the ingredients as percentages by weight follows:

	%
Ground yellow corn	29.0
Finely ground oats	10.0
Ground wheat	20.0
Wheat standard middlings	15.0
Soybean meal	7.0
Fish meal, sardine	3.0
Meat scrap	1.5
Riboflavin supplement	3.0
Alfalfa meal, dehydrated	5.0
Ground limestone	3.0
Steamed bonemeal	2.5
Salt (with manganese and iodine)	0.7
Vitamin A and D supplement	0.3

Such a diet mixture will contain levels of the nutrients generally equal to or in excess of the National Research Council standards. For example, the protein content will approximate 16% in comparison to the 15% required and calcium content will be 2.3% in comparison with the 2.25% required. The principle sources of protein in order are wheat, soybean meal, middlings, corn, and fish meal. Because the concentrate feeds making up the larger portion of the diet are low in riboflavin content, a concentrate rich in this vitamin needs to be added to supply approximately one-third of the full requirement of 1.7 mg./lb. of diet. It is common practice to supply some additional manganese as well as iodine as additives to the salt. Actually, the manganese content of the diet mixture shown above is approximately 16 mg. on the basis of average analyses as compared to a stated requirement of 15 mg. In the case of the added vitamin A and vitamin D, the usual practice is to incorporate a minimum of 2000 U.S.P. units of the former and 225 International chick units of the latter per pound of diet.

The general pattern of chick diets holds true for turkeys also. The number of ingredients is governed in the main by supply, costs, and ability to compound a reasonably complete diet with a minimum number of feeds and feed supplements. Typical formulas for the several classes of poultry diets are described in various poultry feeding handbooks, state and federal experiment stations bulletins, and other channels of publication.

V. OTHER LIVESTOCK

Although the numbers of horses in the United States have declined steadily the past thirty years or more, those still kept for work and pleasure constitute an important segment of the feed-consuming livestock population. Since horses along with mules are fed very little animal-source feeds, the use of plant protein concentrates becomes all the more important.

There are feeding standards for horses just as for other classes of livestock. The energy and protein requirements along with those for some of the more common mineral elements are quite well established. Data for other nutrients are not so numerous. The basic feed of horses and mules is forage. Ordinarily the concentrate feeds are increased in relation to the forage consumption in proportion to the work and exercise performed.

The basal concentrate feeds for horses are oats, barley, and corn, ordinarily rated for desirability in the order named. Of the plant protein concentrates, wheat bran, linseed meal, soybean meal, peanut meal, gluten meal, and cottonseed meal are the more common ones

used. In the usual case, there is little need to add more than one, or at the most two, of these protein concentrates. For example, a grain mixture for a growing horse can consist of two parts by weight of corn, five parts of oats, one part of wheat bran, and one part of linseed meal. Besides a liberal allowance of forage, the animal can be fed a pound of the mixture for each 100 pounds of live weight.

Other classes of animals including goats, fur farm animals, dogs, rabbits, and other laboratory animals such as the rat do not consume the tonnages of feeds that go into the production of those classes already discussed. Nevertheless, all these species consume significant amounts of concentrate feeds, including the plant protein products. Just as for cattle, sheep, swine, and poultry, these last-named products are included mainly to supply protein. A wide choice is generally possible, although the more common oilseed meals and the milling products predominate.

In the feeding of milk goats, much the same practices employed in dairy cow feeding are followed, whereas for angora goats kept on the range, supplementation such as is done in maintaining sheep under similar conditions is followed.

For fur farm animals such as the mink and for dogs, a cereal base containing cereal products and other plant protein concentrates may be included as a part of the diet mixture. Laboratory animals' diets are not unlike swine and poultry mixtures. Rabbit diets frequently contain as much as 40% of alfalfa meal, with the remainder made up of a concentrate mixture largely, if not entirely, of plant sources.

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CHAPTER 9

USE OF PROCESSED PLANT PROTEINS AS HUMAN FOOD

R. F. A. DEAN

I. GENERAL CONSIDERATIONS

1. Early Sources of Protein for Human Food

The forerunners of man, and man's earliest types, must have derived nearly all their protein from animal sources. Many of the stone tools that were the earliest artifacts were undoubtedly made especially for the killing of birds, fish, and other animals, and the tools are often found with the animals' bones.

If we accept the evidence of the Mesolithic (9000 to 4500 B.C.) stone sickles polished by contact with siliceous stems, the first plant foods to be gathered were wild grasses that were the ancestors of modern wheats. The domestication of the grasses may have been coeval with the domestication of the sheep and goat, and perhaps of bovines. According to one view (1) the animals may even have been domesticated before the plants. The cultivators and the animal keepers were possibly separate groups, because the food requirements of more than a small number of animals could only be met by nomadism, and because stability is the first requirement for agriculture. Any attempts at combining plant cultivation with animal keeping would involve immediately a problem still unsolved today—the extent of the competition to be allowed between man and his animals for food itself or for the land on which food can be grown.

Although there is some evidence that corn may have originated from ancestors that would be classed among the high-protein crops—one may have been *teosinte*, the Central American plant whose seeds contain about 24% protein—there is nothing to suggest that any great modification in protein content has occurred in other cereals. The supplementation of cereals by other foods richer in protein is universal practice, and one so constant that an instinctive need might almost be adduced. The inhabitants of some of the earliest agricultural settle-

ments, such as those of the Neolithic culture in Merimde, Lower Egypt, and the Fayum, that may be dated between 6000 and 5000 B.C., grew wheat and barley and kept cattle, pigs, and sheep (2); a wild pea found at Merimde (3) may not have been cultivated, but peas were cultivated in Europe by the beginning of the Neolithic period. Lentils were another crop brought very early under cultivation, probably well before their first record in Khafaje in southern Iraq (about 3000 B.C.). They and the broad (or horse) bean (*Vicia faba*) were established in Europe by the Bronze Age, and have been found in the Swiss Lake dwellings of that age. Zeuner (3) says that, although the domestication of the goat and sheep may have preceded agriculture, it was the cultivation of cereals that made settled life finally possible, and that the pulses, and the rapid growers generally, would naturally receive early attention. In some places, pulses may have followed cereals only after many hundreds of years; the impressions of seeds and grain in Danish Neolithic pottery—the result of the pot being made on the unswept floor—show that wheat and barley were cultivated between 2500 and 1500 B.C., but bean and peas not until 800 to 400 B.C. In other places pulses may have been grown, but no cereals. In Huaca Prieta, on the Peruvian coast at the end of the Chicama Valley, a site inhabited between 3000 and 1000 B.C., at least three kinds of bean (probably *Canavalia* spp.) were cultivated, but no maize (4). Fish was one of the chief sources of food, and the diet must have been unusually rich in protein. Of developments running into modern times Zeuner (3) says, "The high protein-content of pulses has enabled civilization to persist where animal food is scarce, or for religious or other reasons unobtainable. Whole populations in the East are mainly or wholly vegetarian and obtain their protein largely from pulses, such as the soya bean (*Glycine max*) of China and Japan and the grams of India (*Phaseolus mungo*, the black gram, and *P. aureus*, the golden gram)."

2. Competition for Food between Man and His Domestic Animals

So long as domestic animals eat only the grasses and leaves that are unacceptable as food for man, they are not serious rivals for the communal food supply. They become competitors if they have to be grazed on land that is needed for plant cultivation, and the competition is sometimes intensified by the limited distance at which control of either animals or plants is practicable. In the modern world it is of course common practice to use good land, on which food could be grown that is utilizable directly by man, for the growing of food for animals. Although the animals manure the ground they feed on and sometimes

contribute to a more balanced agriculture, the process is wasteful in terms of the protein in their feed that they return for human consumption: the cow yields 23%, the pig 12%, and beef cattle only 10% (5).

The universal desire for animal protein is satisfied only at a price, and when it is realized that the high yield of the domestic animal—the yield that makes mixed farming “pay”—depends in many instances on giving the animal food that is a normal part of the human diet, it becomes important to consider whether or not the price can be afforded. In poorly developed countries, it cannot be assumed without proof that unlimited mixed farming is necessary or desirable. The question will undoubtedly be debated more and more as the world's population continues to rise. The situation will rapidly become critical in India, and in other countries that have small resources of unused land, and a huge population already underfed. Patwardhan (6) has pointed out that, despite all efforts, the milk production of India is not rising, although it is completely inadequate by Western standards. It may be that it cannot rise simply because it cannot command enough land, and enough human food.

It is doubtful if the world's production of protein is keeping pace with its increase in population (7). Protein, especially in concentrated forms, is always expensive, and one reason seems to be that soil more easily runs short of nitrogen than of minerals, or of any other material required for plant growth. A subsistence agriculture has been maintained for centuries in parts of China by the meticulous return of all excreta, of man as well as the domestic animals, to the family plantation. In many countries manuring is supplemented by the use of legumes that live symbiotically with nitrogen-fixing bacteria; in others there is further supplementation with artificially produced nitrogenous substances that are expensive and that may be less effective for many purposes than green manures on the basis of equal nitrogen (8). It is fortunate that many legumes not only enrich soil with nitrogen, but yield large amounts of nitrogen in an edible form; but legumes, like all other plants and animals, must be supplied with the nutrients they require. It is also necessary to realize that the selection of legumes for the tropical countries that are the chief centers of undernutrition has so far hardly begun, and much research is needed to find the varieties that are best suited to conditions of limited and uncertain rainfall, high temperatures, and poor soils. Nevertheless, although other sources of protein will be mentioned in this chapter, it will be obvious that the legumes represent one of the most important sources of protein for the future and that their utilization must dominate any discussion of man's use of plant proteins.

3. Vegetarianism and Vitamin B₁₂

The restriction of human diets to vegetable products, which appears to have been unusual in remote history, probably never occurs except by deliberate choice or extreme misfortune. Many groups of people are unable to obtain much animal food, but no instance is known of a primitive population that has none at all: an enforced period of abstinence is followed by an orgy of meat-eating if the opportunity arises. A vegetable diet supplemented by milk and milk products can be nutritionally adequate in every way; the Hunza tribe of northern India, described by McCarrison (9) as being physically perfect, have such a diet, and modern lactovegetarians in Europe and the United States, although not usually outstandingly fine in physique, find no difficulty in keeping healthy. The rural American community described by Mirone (10) had diets that contained very little animal protein—meat was never eaten, cheese only on Sundays, and milk only in coffee and bread—but the men were capable of sustained hard physical work and the blood chemistry of all the subjects investigated was entirely normal.

Vegetarianism may be a religious tenet, in which case it usually involves no more than abstinence from flesh foods, or it may be followed for various reasons that range from economy to reluctance to inflict pain on animals (11). There are extremists who take no animal food of any kind; they are known as “vegans” and have lately achieved some prominence because some of them show the effects of a deficiency of vitamin B₁₂, a deficiency not previously recognized in man.

Three reports have appeared: by Harding and Stare (12) on 26 white Americans, by Donath *et al.* (13) on 61 Dutch, and by Wokes and co-workers (14) on 83 British. The American vegans were about 20 pounds (9 kg.) lighter in average weight than comparable groups of lactovegetarians and non-vegetarians, but were having an intake of about 3250 Calories daily, and about 80 g. of protein, and their serum cholesterol level was 206 mg. per 100 ml.; the comparable figures for the lacto-vegetarian and the non-vegetarians were 3020 and 3720 Calories, 98 and 125 g. of protein, and 243 and 288 mg. per 100 ml. of cholesterol. The differences in the cholesterol values probably reflected the protein intakes rather than the intake of preformed cholesterol. In the treatment of the protein-deficiency disease of children known as kwashiorkor, a diet providing no cholesterol but rich in protein rapidly raises the serum cholesterol to a high level (15). The British group of vegans contained a larger proportion of young people than the Dutch and had been vegans for an average of between 5 and 6 years, compared with the 2 years of the Dutch: these factors may have been responsible for their more serious symptoms. The chief troubles were sore tongues, parasthesiae, and various nervous disorders, some very serious; two deaths that occurred in the British group were “associated with nervous breakdowns.” In discussing the causes of the symptoms, Wokes and his colleagues made out a good case for

inculcating a lack of vitamin B₁₂, either in the diet or synthesized in the intestine. They also pointed out that, whereas the American vegans were obtaining 10.4% of their total calories from protein, the British, who showed the most effects, were obtaining only 7%. Unpublished data, still being collected, were indicating a subnormal rate of growth in British vegan children, although lactovegetarian children were known to grow at the normal rate. Further investigation is needed; it is not yet known if the untoward effects of a vegan diet can be avoided by adding vitamin B₁₂ to the diet—a solution that might be easier than the conversion of the vegans to lactovegetarians.

The relevance of this diversion lies in the fact that vitamin B₁₂ usually accompanies animal protein but not vegetable protein. (See Table III, page 62.) The amounts of the vitamin contributed by bacterial synthesis in the human intestine may also be important, but cannot, it seems, always satisfy requirements. Until further evidence is forthcoming, it is reasonable to suggest that diets, especially those of young people, should contain some animal protein—a conclusion that very few will find offensive. It is, however, a conclusion that has a bearing on some of the published results of attempts at feeding children on diets from which animal protein was excluded. These results were summarized in an earlier monograph (16) which also contained an account of the author's controlled trials of plant protein diets in infant feeding. Nearly all the experiments were of short duration, lasting only a few months, and it is almost certain that the subjects were stored, before the start of the experiments, with sufficient vitamin B₁₂ to prevent the appearance of signs of a deficiency. It has been left to the vegans to perform on themselves the experiments that physiologists and others would condemn as unethical.

It is unlikely that a simple solution to malnutrition will be provided by the addition to poor diets of vitamin B₁₂, or of antibiotic materials that have an action like that of the vitamin. Various experiments have been made with young children, by the present writer (unpublished) and by other workers (17, 18) but the results have mostly been negative, and the only conclusion that can be drawn is that the amount of the vitamin needed in the diet is small and is usually provided by a very little food of animal origin.

4. Protein Requirements

a. Of Children

The fully breast-fed child can grow perfectly although receiving small amounts of protein. Very few reliable figures for intakes, properly supported by analysis, are available, but Holt,* after a careful

* L. E. Holt, New York University, Bellevue Hospital, New York, personal communication.

study of the literature, has decided that the figure of 2.0 g. of protein per kilogram might be taken as providing an adequate margin. When the child has to be fed on a mixed diet, it is usually considered necessary to increase the proportion of protein. If the diet contains plenty of cow's milk, the desirable figure is probably about 2.5 g./kg., and if the animal protein has to be restricted, so that nearly all the protein must be derived from vegetable sources, it is probably 3.0 to 3.5 g./kg. This amount of protein was taken by children aged 6 months to 2 years in one series of experiments (16) in which 80 and 100% of the total calories were obtained from a mixture of soya, wheat, barley, and vegetables.

Various reasons can be suggested why the mixed diet should contain more protein than breast milk, and it is likely that the most important has nothing to do with the protein itself, or at least with the amino acids that make up the protein. It is not difficult to ensure a reasonably perfect complement of amino acids in a diet exclusively of plant origin, the similarity of amino acid compositions, and not their differences, being remarkable; a cereal and a pulse, for instance, will together compensate for each other's deficiencies and will provide a mixture in which there is probably no shortage of any physiological importance. The way in which the protein is cooked, and the amounts of fat, and of indigestible material, with which it is associated, deserve more consideration. If it is necessary to devise an all-vegetable diet for a child, the first thought should be for the total amount of protein, and afterward, when that has been assured, the manner in which the protein is to be offered. Porridges and much-used staple foods, such as cooked bananas (plantains), potatoes, and cassava roots, are so bulky in relation to the protein they contain that no child can obtain from them all the protein he needs, and when they are given in excess the appearance of the stools, in which large amounts of undigested material can be plainly seen, suggests a great waste of the food value.

Unfortunately, no figures based on the careful experimental feeding of children on various diets can be given for the optimal requirements of protein of any kind, and for the time being it is best to base an estimate on some measured intakes. There are objections to this course, but experience with children usually leads to the conclusion that intakes are approximately the same as requirements, except in pathological conditions in which the appetite is perverted or reduced. One of the most satisfactory sets of values was given by Widdowson, (19), who investigated the diets of British children between 1935 and 1939, and the protein intakes she found are shown in Table I. As about 12% of the total calories was from protein, and as no less than two-thirds of

the protein was from animal sources, it may be well to consider these figures as *minimum* requirements if the diet is much less rich in animal protein. It is realized that this recommendation is far from being attainable in many situations; well-fed boys at a Uganda boarding school, although their ages ranged up to 20 years, were found to be taking less than 60 g. of total protein, which compares unfavorably with the

TABLE I
DAILY INTAKES OF PROTEIN OF BRITISH BOYS^a

Age (years)	Average protein intake (g.)	Range of protein intake (g.)	Average body weight (kg.)	Protein intake (g./kg.)
1-2	37	28-61	11.8	3.1
2-3	41	29-57	13.9	2.9
3-4	49	29-80	15.9	3.1
4-5	52	34-82	17.9	2.9
5-6	50	36-64	20.2	2.5
6-7	55	26-71	21.4	2.6
7-8	63	46-80	24.6	2.6
8-9	60	39-86	25.2	2.4
9-10	68	53-94	30.1	2.3
10-11	73	47-94	31.0	2.4
11-12	72	43-113	35.1	2.1
12-13	76	57-117	37.3	2.0
13-14	79	53-117	45.0	1.8
14-15	89	56-132	49.1	1.8
15-16	100	78-132	54.1	1.8
16-17	94	72-138	61.0	1.5
17-18	95	71-142	65.8	1.4
18-19	97	67-115	65.0	1.5

^a Adapted from E. M. Widdowson, "A Study of Individual Children's Diets," *Med. Research Council (Brit.) Spec. Rept. Ser. No. 257* (1947).

70 to 100 g. of Widdowson's children (20), and 4 healthy Jamaican children aged between 16 months and 6½ years were having only about 28 g. of total protein (21). When it is realized also that the losses of nitrogen in the stools are often much higher in poorly fed subjects than in well fed ones (22, 23) and that the stool nitrogen of non-dietary origin may also be higher, generosity in protein allowances becomes a duty.

b. Of Adults

In contrast to what has just been said about children, experience with adults leads invariably to the conclusion that overeating is ex-

tremely common, and that intakes are a poor guide to requirements. It is possible to find the minimum amount of protein needed to maintain nitrogen equilibrium, or normal levels of nitrogenous body substances, but the results have little practical value; even the upper limit of useful intake is difficult to define because excess protein is utilized as a source of energy. It is usually held that 1 g. of protein per kilogram of body weight is a reasonable adult intake, but at a Conference (held jointly by the Food and Agriculture Organization, the World Health Organization, and the Macy Foundation at Princeton, in 1955) (23a) it was decided that this figure could be reduced safely to 0.6 to 0.7 g., provided that a good mixture of dietary amino acids was being taken. More may be needed in some circumstances, and it seems likely that if the diet is almost entirely vegetable, a higher protein intake—probably 1 g./kg.—may be advisable. (See also pages 16–19.)

II. PLANT PROTEINS NOW IN USE

1. Foods That Can Be Prepared in the Home

a. Cereals

The home production of cereal flours from grain, although largely superseded by commercial methods in advanced countries, is still used in millions of primitive homes. An interesting recent development seen in a rural area of Southern Rhodesia is the taking of home-grown corn grain to the local trader, who gives ground meal for it. Providing that a reasonable proportionality is maintained on the exchange, the practice seems to be worth encouraging; it relieves the women of work that must consume immense amounts of energy. The same advantage was being obtained by the women who were seen by the present writer in Guatemalan hill villages, taking their lime-soaked corn to the village baker, to be ground into a paste by his machinery.

Although cereals are not usually considered as “protein” by the ordinary man, they are often the chief protein source in the diet. In the highly developed countries, the quantity consumed is usually masked by the diversity of the ways in which the cereals are taken, but at the end of a day that includes a normal breakfast (porridge or a proprietary breakfast food, toast), lunch (with bread accompanying the main dishes and the cheese), tea (of cake and biscuits), and an evening meal (with rolls, bread sauce, or breadcrumbs and a cereal pudding), the total is likely to be remarkably high, even if the usual snacks between meals (bread or pastry) are foregone.

Widdowson (19), in the survey already mentioned, found that from the age of 3 years no other item in the diet provided as large a proportion of the total calories as cereals, and that even the group of children whose average age was 18 months were eating about 60 g. of wheat flour, or its equivalent, daily, and boys 15 years old, 240 g. These amounts provided 18 to 25% of the total calories, and 14 and 22% of the total protein calories. In another group of children, who were dealt with separately because their fathers were unemployed and the family's food had therefore to be bought as cheaply as possible, Widdowson found that about 50% more bread was being eaten, and although the diet contained less cakes and biscuits the contribution of cereals to the total calories was high; it cannot be calculated exactly from the figures given, but was probably about 40%.

In underdeveloped countries, the number of foods eaten is always restricted, and excessive reliance is often placed on the chief staple. Thus plantation landowners in Guatemala, and village farmers in Honduras, whose diets were measured by investigators of the Institute of Nutrition of Central America and Panama, took about 75% of their total calories in the form of maize (24), and a similarly high percentage is usual in other maize-eating countries. The same is true of other cereals; rural Hindu families in the Raipur district of the Central Province, India, had 89% of their total calories in the form of rice, and outcastes in Hyderabad (Deccan) had 87% as either rice or millet (25); in western Java, according to data from a survey of small groups by the Institute of Nutrition, Djakarta, the consumption of rice averages 585 g. daily per person, and therefore accounts for about 95% of the total calories (26). These are perhaps extreme examples, but there are large areas of eastern and southeastern Asia where the calories of the diet normally include 70 to 80% from cereals. Even in China and Manchuria, normally regarded as depending largely on the soybean, the percentages, calculated from the foods available at the retail level, were 70 and 78 (26).

In deciding the nutritional value of a diet, and its advantages and disadvantages, it is necessary to consider not merely the staples, but all the other items. Except possibly in the case of corn, which may contain a pellagrigenic factor that becomes of greater physiological importance as the amount of the intake rises, increasing the amount of a cereal need do no harm, provided that the rest of the diet fills all the needs not covered by the cereal.

An illustration was provided by the trial of various wheat flours in the diets of German school children, carried out by the Medical Research Council (27). Bread was given in amounts limited only by the children's appetites and provided up to 75% of the total calories. It was also the chief source of protein, the rest being derived from vegetables and small amounts of meat, fish, cheese, and milk; the four items only yielded 8 to 11 g. of protein daily in a total of

60 to 80 g. The children thrived on the experimental diets; they had been undernourished for some years and were below normal standards for height and weight at the beginning, but they developed excellently in the year of the experiment. At the end of the year, a supplement of reconstituted full-cream milk powder was added to the diets, which were otherwise unchanged, and contrary to what might be expected, no further beneficial effects were observed. Presumably the unsupplemented diet was adequate so that the supplement could produce no extra growth. In the first experiment, one group of children had 70 g. of total protein, and of that, 9 g. from the animal products.

The results suggest that cereals can safely be allowed to supply three-quarters of the total calories and protein in children's diets that yield enough total calories and protein for satisfactory growth. If this appears to be a retrograde conclusion to citizens of the United States or any well-fed European country, it will appear near to the expression of an ideal to those of many other countries.

The danger of using cereals too exclusively becomes greatest when, either for reasons of economy or to provide a bland diet, infants are given porridges or broths made without milk. The protein content of the foods is usually very low in proportion to their bulk and also to their carbohydrates, and a kind of malnutrition occurs, of which the chief features were recognized in the middle of the nineteenth century. An account of the early descriptions of the disease, and of similar conditions that occur nowadays in young children whose diets do not contain enough protein, has been given elsewhere (15).

In Mexico and in other Latin American countries, corn is much used in the form of *tortillas*, flat cakes baked on a hot stove or iron plate.

There are many details of interest in their preparation. The Mexican method is as follows: The corn is placed in twice its volume of a lime solution (approximately 1%), heated to about 80° for half an hour, and left to stand until the following day. It is then drained, and the swollen, soft grains are ground. As in Guatemala, the grinding may be done mechanically, but it is more usual to do it at home, on a stone. The dough is made into thin rounds, which are allowed to dry partly, and are then cooked for about half a minute on one side, turned and cooked for a minute and a half, then turned again and once more cooked for half a minute (28). The aneurin (thiamine), niacin, and riboflavin of the corn are well preserved by this method, but much of the carotene from yellow varieties is lost. There is a large gain of calcium from the lime water, and in three samples of tortillas analyzed, the average calcium content was 111 mg. per 100 g.; in fresh corn meal it was 7 to 9 mg. per 100 g. The analyses showed 5.81 g. of protein per 100 g. of tortilla, whereas the corn had 8.3 g. per 100 g. The tortillas contained about 40% moisture—a slightly higher percentage than most wheat breads (30 to 35% in the United States, 35 to 40% in European countries). Mexicans prefer them slightly soft, but in other countries they are baked hard, and therefore contain less water. They are obviously much more nutritious than the porridges and other corn

preparations used very widely, but they still present a formidable physical problem to the small child who must try to obtain nearly all his protein from them—a necessity often enforced by poverty or ignorance (24).

b. Legumes

(1) *Beans and peas.* The use of beans of one kind or another is almost universal. In some places, such as the Ruanda-Urundi (29), they are the basis of the diet; in others, such as Guatemala, even if they are not the staple food, they are eaten every day; in parts of Uganda they are eaten seasonally, but only because storage facilities are so poor that the amounts grown are limited. A general idea of their distribution can be obtained from the Yearbooks of Food and Agricultural Statistics published by the Food and Agriculture Organization. Spain and Portugal, the Central and South American countries, China, and India are the most important. The Latin countries, Spain and Mexico, are large producers of chick peas, Syria and Turkey of lentils, and Egypt of broad beans; and pulses of many kinds are grown in India. (See also Chapter 26.)

Although many of these foods can be eaten green and immature, as peas and kidney beans are usually eaten in the United States and Europe, they are not then large contributors to the protein of the diet; they become important for their protein when the mature dried beans are eaten. Nearly all can be prepared domestically. They are soaked in water until they have absorbed the maximum amount, a process that loosens the outer skin so that it can be rubbed off easily if desired, and cooking for an hour or so makes them soft and floury. Most beans have a distinctive flavor which, although not usually enough by itself for a sophisticated palate, presumably gives a welcome change from the flavor of the cereal or starchy staple. They can be spiced or salted; some contain fat as well as protein, and in more than one way, therefore, they take the place of the animal protein part of the meal. Although it is more usual for them to be served as a separate side dish, they are sometimes cooked with the staple, and even with the staple and meat or fish.

In India, various species of *Phaseolus* are used for making *gram*. The seeds are cooked more easily if they are split. They are allowed to soak and are mashed into a thin paste, strained by squeezing in a cloth bag, and partly dried, boiled for half an hour, squeezed again, kneaded with hot and cold water, and finally dried completely (30). In parts of India, and elsewhere, grams are mixed with rice and other cereals: the mixtures are apparently well tolerated, even by very young children (31). In some places it is usual to soak the gram (in its husk) and some beans in water for 12 to 24 hours and to tie them in a wet cloth. The bundle is allowed to stand for a day or

two, and most of the seeds germinate. After the husks have been removed, the germinated material is eaten either raw or cooked with spices (6).

One of the most important foods in India is *dhal*. The species of plants most often used are the pigeon pea (*Cajanus indicus*), which makes a light yellow dhal; lentil (*Lens esculenta*), which makes a red one; the black gram (*Phaseolus mungo*); and the green (*P. radiatus*). Making *dhal* is a village industry, and there are dry and wet methods. For the dry method, the peas, after being thoroughly sun-dried, are passed through a mill which has its stones so adjusted that the coats of the seeds are cracked, but few seeds are split. A little oil is then added to the seeds, and they are exposed to the sun, which in drying them loosens the coats. The process is repeated several times, with the millstones moved each time closer together, until finally the seeds are all split and the broken coats can be winnowed away. The principle of the wet method is the same, but the seeds are soaked in water and dried in the sun, which again loosens the seed coats. A dry powder is finally produced; it keeps well and is easily boiled into a soft food, free from hard grains (30).

Beans vary considerably in digestibility, and therefore in the degree to which they are suitable for young children, but there seems to be no doubt that with a little care most of their disadvantages can be overcome. Chick peas, which form a fine paste when they are boiled, have been used successfully for infant feeding in the Lebanon, in a mixture containing wheat flour and soured milk, and in southern India they are mixed with bananas and sugar (31). There are undoubtedly many other possible variants, and it is somewhat surprising that they are so little employed (32). Vincent (29) states categorically that in the Ruanda-Urundi, despite the dependence on beans as a staple, there is no local recipe for a bean "milk"; and the inquiries of the present writer, which have been made in many African countries, have failed to produce any well-authenticated examples. Bean "milks" are not made, apparently, in Central and South American countries, and it might be valuable to know why. The chief reasons are probably the lack of appreciation of the need of the small children for a special diet, the natural reluctance to experiment with the diets of infants, who usually show food intolerance swiftly and unpleasantly, and the conservatism that permits the recognition of a food only if it is in a familiar form. On the other hand, it is likely that experiments have been made from time to time and have proved unsatisfactory, or even disastrous, because of the presence of allergens such as those in the broad bean (*Vicia faba*) and believed to be responsible for favism (33); or there may have been injury from toxic principles such as those found in species of *Lathyrus* (34, 35).

(2) *Groundnuts*. Groundnuts are rich in protein and also rich in oil. They are usually roasted, but are sometimes boiled to make sauces and relishes to accompany the main part of the meal, either alone, or

with meat or fish. The groundnut-fish combination is used by those who can afford it in some parts of Uganda and is delicious. They are not easily made digestible for small children, possibly because of the high content of fiber, or the rather hard skin that surrounds the seed. If they are whole, they always have to be chewed unless they are exceptionally well cooked, and even if they are pounded or kibbled, they are difficult to soften completely. In West Africa, they are ground fine before they are made into soup. In highly developed countries they are known best in the form of a commercial peanut butter. The butter can also be home-made: in Southern Rhodesia the nuts are roasted, skinned, and ground on stones with salt into a fine smooth paste. A tin or jar of the paste, containing perhaps 1 kg., is kept by every housewife who can afford it. The Hausas of the Gold Coast make balls or rings of the paste and fry them (36).

Vincent, according to Brock and Autret (37), has been able to teach mothers to make a kind of milk from groundnuts. The nuts are pounded and mixed with about six times their weight of water. The mixture is filtered through cloth and the filtrate boiled for 10 minutes. The "milk" has been used for supplementing breast milk from the age of 4 months.

(3) *Soybean*. The soybean makes a very large contribution to the diet of Manchuria, China, and Japan, but only because in those countries methods have been found that overcome its disadvantages. It needs special cooking. Although simple boiling is sometimes advocated, even in official publications, it does not make the dried beans easily edible; they remain hard and bitter, and if eaten cause diarrhea. In China, the usual method is to soak the beans, break them up, and make an emulsion. A precipitant, either plaster of Paris or the mother liquor from the manufacture of salt from sea water, is mixed with the emulsion, and the mixture is heated. It is the precipitated and filtered "soya curd" that is eaten. (See also Chapter 11.) When fresh, it contains 8% protein, 3% fat, and 1.5% carbohydrate; if it is dried and smoked, it contains about 17% protein (38). The method has various advantages besides procuring edibility and the removal of the bitter taste. It is probable that it removes or destroys the trypsin inhibitor present in raw soya, and it adds calcium.

A somewhat similar procedure is followed in the preparation of *tofu* in Japan; 160,000 tons of soybeans were set aside for this product in 1957. Magnesium or calcium salts are the precipitants of the curd from the soybean milk; the product is eaten by nearly every family in Japan with its breakfast miso-soup (38a).

In more or less primitive conditions, the preparation and cooking of food presents almost as many problems as its cultivation and storage.

It is therefore quite certain that the Oriental method just described is not unnecessarily complicated. During World War II, attempts were made in several countries to introduce soya as a food crop. In Uganda, no instruction was given in the necessary details of preparation, with the result that the crop was very reasonably declared inedible by the Africans. They retain a violent prejudice against it and are suspicious that it has been added to any food, such as yellow corn meal, that they find distasteful.

One of the most interesting methods for making soya edible has been evolved in Indonesia and was described in full by Van Veen and Schaeffer (39). It takes advantage of the ability of the mold *Rhizopus oryzae* to grow on the bean and to alter its constituents. The same process can, it is said, be used on cocoanut and peanut press cake, the fungus *Neurospora sitophila* being used sometimes for the peanut cake. The product made from soya is called *tempeh kedele* (kedele = soybean).

The beans are soaked in water overnight and cooked for a short while; they are then inoculated with the fungus and wrapped in leaves, and the bundle is put in a sheltered corner. In 18 to 48 hours the mass of beans is penetrated and overgrown by a dense network of white mycelium, and it is then ready to be eaten. It is never eaten raw, but roasted, cooked in soup, or fried in oil; it can also be sliced and dried.

In experimental laboratory production, Van Veen and Schaeffer found that 200 g. of raw beans gave 264 g. of *tempeh*. The preliminary soaking and cooking removed about 30% of the original amount of the raw beans, and nearly all the soluble carbohydrate was lost at this stage. The hemicelluloses of the beans were decreased greatly by the fungus, but there was more cellulose in the *tempeh* than in the raw beans because it made up most of the mycelium. The fat was slightly reduced in amount and altered qualitatively, having many small, solid particles instead of being a clear oil, and more than half of the original protein was broken down to water-soluble forms. The method seemed to be an excellent way of making the bean nitrogen easily available, and some experiments with pancreatic enzymes, although not specifically designed for the purpose, indicated that the trypsin inhibitor had probably been destroyed.

One of the fermented soy products of Japan is *natto*; its preparation, as described by Hayashi, is similar to that of the above-mentioned product, the difference being in the organism used for fermentation. It is reported to contain approximately 18% protein, 9.7% fat, 0.8% fiber, 7.7% carbohydrates, and 2% ash (38b).

c. *Sunflower Seed*

The kernels of the seeds of the sunflower (*Helianthus annuus*) are good eating, even raw, and travelers in Russia are familiar with their use as a food for a train journey. The seeds are cracked in the teeth.

and the outer skins rejected, forming a cone between the feet of the traveler; when the cone reaches knee height, the traveler changes his seat.* Large quantities of sunflower are grown, and used domestically, in Rumania, Bulgaria, and Southern Russia. The crop has recently become popular in villages of northwest Uganda, where the seeds are beaten in a mortar and the broken husks tediously picked out by hand. Unfortunately, it is not easy to see how the process can be greatly shortened without the introduction of machinery: if a cheap hand mill could be devised that would remove the husks efficiently, the gain would be great.

d. Sesame

Sesame seed contains 20% protein. It is used as a food by various peoples, including the Karamajong of northeastern Uganda and the Zande of southwestern Sudan. The Zande steep the seed in water for a few minutes and pound it lightly to loosen the coat; it is then thoroughly dried in a potsherd over a fire, with constant stirring, and the coat is sieved or winnowed away. The seeds are roasted again and ground to a paste, which is sometimes added to dried pulses and boiled again to make sauce to accompany the staple foods, and sometimes used as a sauce or broth by itself. Culwick (40) found that in the five months of the year in which the seed was usually eaten by the Zande, the average amount for each person daily was only 18 g. The people of some smaller tribal units under Zande rulers, the Balanda, ate about three times as much and must have obtained about 10 g. of protein daily from it. The amount eaten is probably limited by the tendency of sesame to be purgative—a tendency that may be more serious for children than for adults.

The use of sesame as a sweetmeat or condiment is fairly widespread in the Near East. A sweetmeat called *tahiniya* or *tahina* is made in the Gezira by cooking the roasted seeds in sugar; sometimes the seeds are crushed before the cooking, and sometimes not (41). To make the condiment, the seeds are soaked in water for 24 hours, drained and left to dry for about 8 hours, hulled, put into a strong salt solution, rinsed in water, roasted, and ground between stones.† In diets in which the total protein from other sources is small in amount, the contribution of the condiment may be important.

The composition of some cereals, legumes and other foods is given in Table II; that of some soybean products is shown in Table III.

* H. C. Chick, Lister Institute, London, personal communication.

† A. G. Van Veen, Nutrition Division, FAO, Rome, personal communication.

TABLE II
COMPOSITION OF SOME CEREALS, LEGUMES, AND OTHER FOODS^a
(All values for 100 g. edible portion)

	Protein (g.)	Fat (g.)	Carbo- hydrate (g.)	Calories	Calcium (mg.)	Iron (mg.)
<i>Cereals</i>						
Cornmeal, whole	9.5	4.3	72.9	356	7	2.3
Cornmeal, fine	8.4	1.2	77.8	363	5	1.2
Millet (unspecified)	9.7	3.0	74.1	340	30	4.0
Rice, parboiled	7.1	1.1	78.0	359	14	1.0
Rice, milled, white	6.7	1.7	78.9	360	10	0.9
Sorghum (unspecified)	10.1	3.3	73.8	343	39	4.2
Wheat flour, whole meal	12.2	2.3	71.8	334	36	4.0
Wheat flour, low extraction	10.9	1.1	75.8	364	16	1.0
<i>Other foods</i>						
Bambara groundnut (<i>Voandzia subterranea</i>)	17.7	6.3	61.7	365	90	4.0
Beans, broad (<i>Vicia faba</i>)	23.4	2.0	60.2	343	90	3.6
Beans, field (<i>Dolichos lablab</i>)	22.8	1.0	62.1	340	92	4.6
Beans, lima (<i>Phaseolus lunatus</i>)	19.7	1.1	64.8	341	84	5.2
Beans, mung and urd (<i>Phaseolus mungo and aureus</i>)	23.9	1.3	60.4	340	145	7.8
Beans, sword (<i>Canavalia ensiformis</i>)	22.0	3.9	53.0	391	141	7.4
Brazil nut (<i>Bertholletsia excelsa</i>) ^b	14.0	66.0	11.0	694	—	4.0
Chick peas (<i>Cicer arietinum</i>)	20.1	4.5	61.5	358	149	7.2
Cowpeas (<i>Vigna spp.</i>)	23.4	1.8	60.3	342	76	5.7
Groundnuts (<i>Arachis hypogaea</i>)	25.6	43.3	23.4	546	52	1.9
Lentils (<i>Lens esculenta</i>)	24.2	1.8	60.8	346	56	6.1
Peas, dried (<i>Pisum sativum</i>)	22.5	1.8	62.1	346	64	4.8
Pigeon peas (<i>Cajanus indicus</i>)	20.9	1.7	62.9	343	129	5.8
Sesame seed (<i>Sesamum indicum</i>) ^b	20.0	50.0	10.0	570	1500	15.0
Sunflower seed (<i>Helianthus annuus</i>) ^b	27.0	45.0	14.0	569	100	7.0

^a Unless otherwise indicated, values are from: C. Chatfield, "Food Composition Tables," *Food and Agr. Organization U.N. Nutritional Studies No. 3* (1953); "Food Composition Tables—Minerals and Vitamins," *ibid. No. 11* (1954).

^b Taken from L. Nicholls, "Tropical Nutrition and Dietetics," Ballière, Tindall and Cox, London, 1951. 2nd ed.

2. Plant Foods Used after Factory Processing

a. Cereals

The subject of the commercial preparation of cereal flours is outside the scope of this book. It is important, however, to remember that the flours, made at home or commercially, may provide a large proportion of the total protein of the diet.

TABLE III
THE COMPOSITION OF SOYBEANS AND SOYBEAN PRODUCTS^{a,b} (*Glycine max*)
(All values for 100 g.)

	Water (g.)	Pro- tein (g.)	Fat (g.)	Carbo- hydrate (g.)	Cal- ories	Cal- cium (mg.)	Iron (mg.)	Yield ^{c,d} (kg.)
Dried mature beans	7.5	34.9	18.1	34.8	331	227	8.0	—
Flour, full fat	9.0	35.9	20.6	29.9	347	195	12.1	0.95
Flour, low fat	11.0	44.7	1.1	37.7	228	265	13.0	0.84
Milk	92.0	3.2	2.0	2.3	37	22	0.6	7.5
Curd ^d	85.1	7.0	4.1	3.0	71	100	1.5	3.5
Fermented beans (Chinese) ^d	45.0	17.0	10.0	6.0	—	100	3.7	2.0

^a Unless otherwise specified, values are from B. K. Watt and A. L. Merrill, "Composition of Foods—Raw, Processed, Prepared," *U.S. Dept. Agr., Agr. Handbook* No. 8, Washington, 1950.

^b See also Tables IV, V, VI, and VII in Chapter 14.

^c Yield from 1 kg. of beans.

^d Taken from C. Chatfield, "Food Composition Tables," *Food and Agr. Organization U.N. Nutritional Studies* No. 3 (1953); "Food Composition Tables—Minerals and Vitamins," *ibid.* No. 11 (1954).

b. Legumes

(1) *Bean flours*. The making of bean flours involves soaking, the removal of the outer skins, and some form of cooking, as in the domestic processes for preparing the beans. There is also, however, the need to dry and to grind the cooked beans. The drying must be done with due precautions against damage. (See Chapter 5 for a discussion of heat damage to proteins.) Pea and bean flours are made in many countries, and in parts of Brazil a flour is made from the Brazil nut. The South African Council for Scientific and Industrial Research has announced (42) that in the National Nutrition Research Institute at Pretoria attempts are being made to produce a cheap but nutritious infant food with dried beans as its basis, and to make the beans available in the form of a precooked powder, for incorporation into any ordinary diet. One of the great advantages of such a scheme is that it employs a food in everyday use, and therefore prejudice is not likely to arise, as it must if new foods are introduced.

(2) *Groundnuts*. The groundnut is a high-protein food that does not need elaborate processing. It is possible that appropriate heat treatment will give a product of higher nutritional value than the ordinary home methods (43), but the gain may not be of great physiological importance, even though the raw beans contain a trypsin inhibitor (44).

Removal of oil from groundnuts is described in Chapter 16. Often the oil is removed partially by mechanical means, the remainder being obtained by solvent extraction. Some solvent remains in the residual cake and has to be removed either by steam heat, which may damage the protein, or by dry heat, which is less harmful (45). The cake contains about 50% protein and should be of great value in human nutrition.

A groundnut flour intended for that purpose is being made in northern Nigeria. The cake is dried at a low temperature and milled to produce a flour with a particulate size of 20 microns; it has 48% protein and 5% fat. Production has so far been enough only for a limited number of clinical trials, but the meal is well accepted and satisfactorily digested by young children. "It mixes well, and is liked, in the local gruels and porridges, thickens soup, and can be added to white wheat flour up to 15% without upsetting the bakers."* Autret and Van Veen (46) mentioned the Nigerian production of a similar flour from ordinary press cake that had been sifted by a special process and was free of silica and cellulose, and was being incorporated with various foods. They said also that dried biscuits had been made from a mixture of peanut and maize flour, and that a "milk" had been given to children aged 4 to 6 months. Biscuits intended for school meals have also been made in Uganda, their ingredients being the finely ground nuts, corn and wheat flours, sugar, and cottonseed oil. One-eighth of the total calories is derived from the mixture of proteins, and the cost of the ingredients for 1 kg. of biscuits, which contains 135 g. of protein, is 15 cents (United States). The program of the Pediatric Department of the African Hospital, in Dakar, French West Africa, includes the use of a groundnut flour, sometimes with millet flour and dried skimmed milk, as a supplementary food given out at Child Welfare Clinics in the town.† The groundnut flour was made by a local commercial firm, and it was not possible to obtain the full details of its processing. In countries such as Nigeria and French West Africa, where groundnuts are abundant, the price of the flour can be low; Autret and Van Veen say that the Nigerian price is 8 cents (United States) for 1 kg.

Another line of approach has been used in India. Desikachar, *et al.* (47) made a fine powder of the kernels of the nuts, having first removed the skins; then they added water, boiled, and strained the liquid through a cloth. A similar liquid was made from germinated nuts (48). It was thick and "reminiscent of buffalo milk in appearance," pleasant in flavor, and contained 3.3% protein and 4.2% fat. It was very poor in calcium and curdled when even very small amounts were added. The difficulty was overcome by adjusting the pH to 6.6 with sodium bicarbonate and adding sodium citrate and disodium phosphate, followed by calcium gluconate. The "milk" then contained 61 mg. of calcium per 100 ml.—twice as much as human milk and half as much as cow's milk. A further development (49) was a "milk" made from three parts of ground nuts and one of soybean, both germinated.

* B. N. Nicol, West African Institute for Trypanosomiasis Research, Kaduna, N. Nigeria, personal communication.

† J. Senecal, Hospital Africaine, Dakar, French West Africa, personal communication.

The Indian workers (50) have reported the results of an experiment in which they gave curd, made from groundnut milk, to girls 4 to 11 years old in a Mysore orphanage. The children were divided into two groups, each containing 21 girls, and were offered diets that provided about 1300 Calories daily, but only very small amounts of meat and milk. The curd was given to one group, and extra carbohydrate (of the same caloric value) to the other. The curd group therefore had each day an extra 13.2 g. of protein, 17.8 g. of fat, and 0.37 g. of calcium (giving them a total of 35.4 g. of protein, 26.8 g. of fat, and 0.71 g. of calcium). As might be expected, the group given curd grew faster than the other group and after 6 months had increased by about 2.5 cm. in height and by 1.1 kg. in weight. The results would have been more satisfactory if the gains of the control group, which was identical in age and clinical condition, had not been so poor, and if it had been possible to say whether the extra protein, fat, or calcium was responsible for the improvement of the curd group. All the children in both groups were probably undernourished, before and during the experiment, and if the supplement had been completely adequate the gains might have been spectacular.

The All-India Institute of Science in Bangalore and the Central Food Technological Research Institute in Mysore have recently combined in an attempt to make a high-protein food that can be used to improve any poor diet. It is based on groundnuts, but the press cake made locally is of such poor quality that it has been necessary to arrange for a special production using selected kernels from which the cuticle has been removed after gentle roasting. The use of sound kernels, and the removal of the cuticle, have been found to improve the keeping qualities of the cake. A flour made by grinding the cake has been mixed with roasted Bengal gram (*Cicer arietinum*) and roasted black gram (*Phaseolus mungo*), and the flavor is said to be excellent. The Bangalore Institute has also made a food from a groundnut cake flour, sesame seed cake flour, soybean flour, wheat flour, tapioca, and salt, and found that it made an acceptable and useful addition to the diets of children.*

Details of a suggested plan for the manufacture in India of a cheap food in which most of the protein is from groundnuts have recently been announced (*Food Science* (Mysore), **6** (4), 75 (1957)). The groundnuts are toasted and carefully pressed, and the white meal obtained (containing 8 to 10% oil) is lightly roasted. Three parts of the product are mixed with one part of a flour made from roasted Bengal gram, and vitamins A and D, and calcium phosphate, are added. The costs of production and distribution have been estimated, and it is thought that the finished article (which should contain about 42% protein) might be sold at the retail price of 75 *naye paise* (= 17 U.S. cents) for one pound.

The formula was chosen after acceptability trials, which included the use of sesame meal. The meal, even when only one part was used to nine parts of other meals, gave a taste that was disliked. The value of the mixture of groundnuts and gram, made on the laboratory scale, was tested, with favorable results, in orphanage children and in experimen-

* A. G. Van Veen, Nutrition Division, FAO, Rome, personal communication.

tal animals, and (after some dried skimmed milk had been added) was used for the successful treatment of three children suffering from kwashiorkor.

(3) *Soybean*. Reference has already been made to the disadvantages of the soybean as a human food. The chief is that ordinary cooking is not enough; even if it is prolonged, it does not remove the bitter taste or destroy the trypsin inhibitor. (See Chapters 5 and 14 for a discussion of the trypsin inhibitor.) The bitter substances can, however, be removed by steam heating; some are volatile and escape with the waste steam from the heating chamber, and steam heating will also destroy the inhibitor, providing that it is done at a little more than atmospheric pressure. The heating must not be carried on too long, or at too high a temperature, because it will then cause deterioration in the quality of the protein. On the other hand, heating insufficient to destroy completely the inhibitor may inactivate so much of it that the residual amount is unimportant, or may alter it so that it becomes susceptible to peptic digestion. One experiment (51), in which two men were given 75 g. of soya protein as soya flour which was raw or had been autoclaved, indicated better nitrogen retention from the autoclaved flour (although there was no improvement in digestion or absorption of the protein), and, especially for children, adequate treatment of the bean appears to be advisable.

As with nearly all dried beans, soybeans can be soaked until they have taken up as much water as they can carry, and the skins can then be rubbed off and washed away. In the commercial production of soya flours, the preliminary soaking may be omitted, the beans being steamed directly. In one process the steaming is interrupted after 15 minutes, and the beans are dried and cracked, the husks removed, and the kernels ground and steamed again. This method is probably more effective than continuous steaming of the intact bean. The whole of the soya oil can be retained, or part or all of it can be extracted. If a half-fat flour is wanted, mechanical pressure is usually employed, and for a low-fat flour, continuous solvent extraction. Great use of soya flour was made in Germany and in German-occupied countries of eastern Europe during World War II, and in Britain at the same time it was used to "extend" meat, in sausages and patties, but with the reversion to normal diets after the war the bulk of the flour was used for animal feeding.

Soybean flours can be mixed with cereal flours and baked into bread and biscuits, and it is likely that all vestige of the trypsin inhibitor disappears in the process. The incorporation of at least a small amount of the flour into cereal flours of any kind seems to present no difficul-

ties and is in theory unobjectionable; experiments have several times been reported (but without details sufficient to indicate the kind of flour and its preparation) from several countries, and the protein value of the soya seems to have been high. Difficulties arise when attempts are made to prepare soya so that it can be given to children of all ages, including the newborn.

The early attempts to use soya for infant feeding have been summarized in a monograph (16), from which Table IV (52-74) has been taken. It will be seen that various preparations have been used, some based on soya flour, and that the making of spray-dried soya "milks" has a history going back many years. The milk is the form in which soya, as a food for infants, is best known in the United States, where it has been developed largely for children who are allergic to cow's milk. There is evidence that the effects of sensitivity to some proteins are seen most readily in very young children, in whom the intestinal canal is permeable to the passage of intact proteins, and that a child who is likely to develop a specific sensitivity—for example a child with a strong family history of allergy—may benefit by being given in infancy a diet from which the allergen has been excluded (75). In clinical pediatrics, cow's milk is the most common allergen, and the continually increasing number of failures of breast feeding must be giving rise to more and more cases of allergy. A number of milks, some in a liquid form and ready for use except for the need to dilute them with water, and others as spray-dried powders that must be mixed with milk, are now on the market; one of the liquid preparations was used by Glaser and Johnstone in the most thorough experiment so far reported. Sixty-seven infants received the milk, at first thinly diluted and afterward at the normal strength; the growth of most of them was satisfactory, but nine had to be taken off the diet, the chief reasons being diarrhoea, vomiting, and colic. Other children had diarrhoea or vomiting or colic probably due to the soya, but not so badly that their diet had to be changed.

Although allergy to soybean is believed to occur (76), Glaser and Johnstone were not altogether satisfied that an allergic response was the cause of their failures, and very reasonably suggested other causes, such as the physiological and anatomical immaturity of the intestinal tract, and the apprehensiveness of the mothers, well known by pediatricians to be infectious and to cause gastrointestinal disturbance in children. Their experience agrees, however, with that of the present writer, and with that of other doctors of his acquaintance who have used soya extensively; although the majority of children of all ages will tolerate soya well if it is properly prepared, a few children in any

TABLE IV^a
INGREDIENTS AND COMPOSITION OF SOYA PREPARATIONS, WITH DETAILS OF THEIR PRACTICAL APPLICATION IN CHILD FEEDING

No. of prepara- tion	Ingredients and method of preparation	Protein (%)	Fat (%)	Carbo- hydrate (%)	Details of use in child feeding, with comments	References
1	Beans were soaked in water overnight, de- husked, and ground to a paste, which was filtered. The filtrate was boiled for 20 min- utes before use.	4.4	1.8	1.5	One child 5 weeks old who was given the milk for 8 months developed rickets because the calcium content was too low, but otherwise it progressed satisfactorily. The Calorie in- take was from 110 to 130 per kilogram body weight.	52-57
2	As No. 1, but filtrate was boiled for only 15 minutes. Cane sugar, egg yolk, sodium chloride, and calcium chloride were added, and the mixture was spray-dried.	29.0	16.0		Given to a child 7 weeks old, who grew satis- factorily.	58, 59
3	As No. 1, but filtrate was boiled for 60 min- utes. Cane sugar, bean starch, sodium chlo- ride, and calcium lactate were added. The mixture was heated again before use.	2.5	1.6	6.8	Given to 15 children from 2 to 6 months old. Development was good, but 9 children had mild rickets.	60
4	Beans were roasted and ground. Method otherwise as No. 3.	4.2	1.4	10.2	Given with vitamins A, D, and C to about 50 children, it seemed to be a complete food.	61
5	Beans were roasted and ground. Wheat, sugar, bone meal, and sodium chloride were added, and the mixture autoclaved.	4.0	1.9	11.0	Theoretically an excellent mixture in the preparation of which the principles of pro- tein supplementation and thorough cooking were combined. Some damage to the protein may, however, have been caused by roasting.	62
6	To a milky fluid obtained from beans "spe- cially treated to remove unpleasant flavors" (no details given) were added malt syrup, lactose, cottonseed oil, butter fat, cod-liver	14.0	18.0	53.0	A mixture devised after detailed consideration of the properties of soya. It was given to about 200 children. As the sole food of children it produced variable gains; older	63, 64

children given it as a supplement to breast milk grew better, but not as well as children given breast and cow's milk. The stools were frequent, bulky, and green, and contained large food residues; they improved if the mixture was boiled. The substitution of 7% milk protein for 7% of the soya protein did not seem to improve growth.

65-71

A commercial preparation, sold in the United States, resembling a mixture devised by Hill and Stuart which gave good results in infantile eczema. The original mixture contained calcium carbonate, but Stearns advocated the use of dicalcium phosphate instead. Hill's results were confirmed by Ribadeau-Dumas *et al.*, who found, however, that the weight gains were not always satisfactory. Klein also recommended the mixture, but said children tired of it. For the use of soya in eczema, see also Mader and Becker.

This was another United States commercial preparation sold for the treatment of allergic conditions in children. It differed from No. 7 in being a wet material and in having much more fat.

72, 73

In a series of trials about 80 children under 4 years old and 60 from 5 to 10 years old were given the milk. The results could not be treated statistically, but it was considered

oil, vitamin C, iron and calcium glycerophosphates, sodium and potassium chlorides, magnesium and calcium lactates. The mixture was homogenized and spray-dried.

7 Soya flour (61%), arrowroot starch (9%), dextrimaltose (6%), olive oil (19%), dicalcium acid phosphate (4%), and sodium chloride were mixed with water, homogenized, dried, and tinned.

8 Soya flour (12%), soya oil (6%), dextrose and sucrose (5%), soya lecithin (0.2%), calcium phosphate (6.7%), calcium carbonate (0.2%), and sodium chloride (0.4%) were mixed with water (75%), homogenized, and canned (tinned).

9 Soya flour was mixed with five to six times its own weight of water containing sodium bicarbonate, sodium citrate, sodium chloride, and disodium hydrogen phosphate (0.1% of

TABLE IV (Continued)

No. of prepara- tion	Ingredients and method of preparation	Protein (%)	Fat (%)	Carbo- hydrate (%)	Details of use in child feeding, with comments	References
	each). The salts were used to help in bring- ing the solids into solution. The mixture was shaken, allowed to stand overnight, and filtered. The filtrate was boiled for 15 minutes.				that the weight gains compared favorably with the gains of similar children given cow's milk.	
10	Equal amounts of soya flour and full-cream, dried milk were mixed together. Sugar and hot water were added, but the mixture was not boiled.				Eighty children from 1 to 5 months old were given the mixture. Their stools were more frequent, and softer, than those of a com- parable group given cow's milk without soya; 19% of the soya group, but only 6% of the cow's milk group, failed to gain weight and had gastrointestinal upsets. The stools improved if the mixture was boiled.	74

^a Taken from R. F. A. Dean, "Plant Proteins in Child Feeding," *Med. Research Council (Brit.) Spec. Rept. Ser. No. 279*, 1953.

large series will react adversely. One especially clear example was a child who, although apparently perfectly willing to take a soya drink and ready to enjoy it, invariably vomited it all within a few minutes; he did not vomit cow's milk taken in exactly the same way (16).

Preparations such as the liquid one used by Glaser and Johnstone are usually made from soya flour, soybean oil, sugars, and salts, steam-heated and homogenized. Other commercial products are slightly different in principle, in that they are a "milk" that is really a suspension of finely ground soya, steam-heated (with reduction in pressure from time to time to allow the escape of volatile substances), with soya oil, sugar, vitamins, and minerals added, homogenized, and spray-dried. The dried "milk" so made is easy to reconstitute, has itself a good flavor and can be given the flavor of malt or chocolate, and is accepted readily by children. It has, unfortunately, the great disadvantage that it is necessarily expensive. The cost of even a simple spray-dried process is high; the spray-dryer is undoubtedly the best dryer known, from many points of view, but also the most expensive to operate, and in the making of a powder such as that described, the yield from the starting material is certain to be low. In a batch of spray-dried soya milk made for the writer, the beans gave only one-seventh of their weight in powder. The powder was excellent in every way and could be given safely to ill children whose digestive powers had been severely impaired, but it was not regarded as a practical solution to local problems of undernutrition (77).

It seems to be necessary to evolve some cheaper method, preferably one using the whole bean. In Uganda, laboratory preparation of soya has been carried out in this way (32). The beans were soaked in water overnight and rubbed to remove the husks, which were washed away with water. They were then drained, minced, and packed into jars of the kind used for preserving fruit. The jars, with their tops loosely screwed on, were placed in a large cauldron filled with water, and boiled for 8 to 10 hours. At the end of that time the beans had become quite soft and had lost all their bitterness, and could be pressed easily into a thin paste between thumb and finger. In the sealed jars, they kept indefinitely, but for large-scale distribution a drying process would have to be added. Sun-drying, followed by grinding to a fine flour, might be practicable, but has not so far been tried. It is obvious that one of the important considerations in such a method, unless it uses the heat of the sun, must be the cost and availability of power or fuel. In Uganda, which has no oil or coal, there is ample electric power, but heating by electricity is often found to be prohibitively expensive in food processing.

A screw-pressed soybean flour is the chief ingredient in the "Multi-Purpose Food" distributed by an American organization. In one formula the flour is salted, seasoned, and spiced; in another, which is intended especially for infants and for use in hospitals, one-quarter of the soybean protein is replaced by dried milk. The makers recommend that for infants the food should be

boiled in water for at least 10 minutes and then strained, and that only the strained juice should be given. Large quantities of the food have been exported to many countries. The results are said to have been satisfactory, but no controlled trials, in which the food has been compared with foods known to be of high value, appear to have been reported.

(4) *Combinations of soya and cereals.* Combinations of soybean with cereals have many times been advocated, chiefly because soya contains large amounts of the essential amino acid lysine, and the cereals are relatively rich in methionine. One of the first examples of the use of the combination appears to have been inadvertent. Rittinger and Dembo (see item 6, Table IV), who made an excellent study of soya milk in infant feeding, were the first to show in a large number of cases that it could be safely and advantageously used. Their mixture included malt syrup, which probably contained considerable amounts (about 10% of its solids) of cereal protein. Willemin (78) also used malt, in a gruel that was made from 10 parts of cream of rice, 5 parts of sugar, 4 parts of soya flour (solvent-extracted), and 2.5 parts of malt extract. She also was not intending to use the supplementary effect, but to reduce the gruel, by the diastatic action of the malt, to a thin liquid that could be taken easily by very young children. She found that the gruel was good for the treatment of diarrhoea, but that children did not gain weight satisfactorily until some cow's milk was added. She noticed that many children disliked the taste, a difficulty that had been foreseen many years before (79).

In the trials carried out in Germany by the present writer (16) cereals and soya were put together deliberately. One of the mixtures used was made as follows: 89 kg. of barley malt flour and 11 kg. of wheat flour of 70% extraction were mixed with about 450 liters of water, and the temperature was raised to 50° and maintained there for 45 minutes. The temperature was then raised to 70° until the iodine reaction was negative. The soybeans (100 kg.) were steamed for 50 minutes, dried, ground, and sieved. Forty kilograms of a fine powder was obtained; it contained 41.5% protein and 20.9% fat. The powder was suspended in water and added to a sufficient quantity of the malt suspension to provide 80 kg. of malt solids. The whole was concentrated to about 75% solids in a vacuum pan, diluted to a consistency suitable for spray-drying, put through a hair sieve, and spray-dried. The powder so obtained contained 17.6% protein and 7.5% fat, and 100 g. provided 423 Calories. It had insufficient calcium (211 mg. per 100 g.), and 3 g. of calcium lactate (= 577 mg. Ca) was therefore added to each 100 g. before use. At the time when the powder was first made, the importance of the trypsin inhibitor was not fully realized, but it was likely that the preliminary steaming was sufficient at least to cause some modification of it. The results obtained by incorporating the mixture in the diets of children aged 1 to 2 years were good; although the diets included no milk or animal protein of any kind, the weight

gains and the clinical improvement over 16 weeks were almost the same as those in a comparable group of children whose diet was identical except that full-cream milk powder replaced the soya-cereal powder. In another trial, for which the soya-cereal powder was made with soya that had been steamed long enough to destroy all the inhibitor, the weight gains were even better than before and exceeded those of children given the cow's milk diet. The manufacturer experienced considerable difficulty in making the soya-cereal powder. The mixture could be spray-dried properly only if the dilution and the intake to the dryer were very carefully controlled after numerous empirical tests had shown the best ways, and darkening in color occurred if even slight overheating was allowed. The dark material was found in clinical use to cause diarrhoea, and more than one large batch had to be rejected because of this fault.

c. Sunflower Seed Meal

The simple pressure extraction of the oil from sunflower seeds leaves a mass of debris in which the hard shells are inextricably mixed with the remains of the kernels. If, however, the seeds are decorticated and the thin skin removed, the oil can be extracted with moderate pressure (and little rise in temperature) or by solvents. If the residual cake is dried and passed through a suitable mill, a fine flour can be obtained. After pressure extraction, oil is usually left in such quantities that the flour does not keep well; but after solvent extraction the keeping qualities are greatly improved. (See Chapter 19.)

Willemin (78) made the most extensive trial of sunflower meal in the experimental feeding of children that has so far been reported. She used a meal that had been extracted with petroleum ether, and of which 60% of the dry weight was nitrogenous substances. She followed the exemplary course of running preliminary animal experiments to find out the biological value of the meal. They showed that the meal, with a salt mixture and vitamin A, would not support the growth of recently weaned rats, and neither would the same diet with enough sugar to reduce the protein calories to 26% of the total, but that the mixture of the flour and sugar and a little milk gave good growth. Later she obtained good results by using lactalbumin instead of the milk, a success that made her decide that the poor quality of the protein was the chief fault in the sunflower meal. A further experiment showed that yeast was also an effective supplement, and it now seems possible that some of the failures may have been due to shortages of vitamins.

In the circumstances, it must have required some courage to carry out a program of trials with sick children. The trials were uniformly successful, however, in that the diarrhoea that was the chief symptom was nearly always arrested, and the conclusion was reached that for the treatment of diarrhoeic conditions a sunflower diet was an excellent

choice. Willemijn found that prolonged boiling, although advisable to render the meal easily digestible, caused the formation of large clumps, but that this tendency disappeared if a little starch was added. She needed a thin liquid that could be given by bottle, and therefore decided to use malt, which she had also used in her soya mixture.

Her formula consisted of 10.0 parts of rice starch, 5.0 of sugar, 2.5 of malt, and 4.0 of the meal (salted with 2.5% sodium chloride). The starch and the meal were mixed in water, brought slowly to a boil, and boiled with stirring for 20 minutes. The resulting gruel was allowed to cool, and the malt was added; liquefaction occurred rapidly, and the diastatic action was then stopped by boiling for another minute. Finally, the liquid was sugared. The ingredients were so chosen that 1 liter provided 700 Calories, and 14.5% of the calories were from protein. The "milk" was given to over 100 children, some of whom were only 6 weeks old, and the oldest only 2 years. It was usually taken readily; the stools had a peculiar and characteristic odor, like that of a henhouse, and were rather bulky, but were otherwise good. The arrest of diarrhoea was dramatic, but the weight gains during convalescence were poor. To overcome the supposed amino acid deficiency, the protein value of the diet was raised so that 40% of the total calories came from the meal, and with this alteration weight was gained well. No ill effects were seen from the high level of protein, but it was considered more practical to use other measures for ensuring good growth, and a small amount of cow's milk or yeast was usually added instead of the extra meal. A regime was adopted for most cases in which cow's milk was gradually substituted for all the meal.

There was another feature of this comprehensive study. A mixture was made of potato flour (50 parts), sunflower meal (50 parts), and malt (20 parts) that, when cooked and sugared, provided 565 Calories in each liter of gruel, with 21% of the calories from protein. Willemijn suggested that it would be an excellent supplement to the low-protein diet of children in poor circumstances, and she was probably right. It would be valuable to try a dried preparation made from these ingredients, but the cost would probably be rather high because the malt would almost certainly be expensive. As with the soya mixture in which malt was used, the malt may have conferred more advantages than mere liquefaction, and it would be necessary, if the work was repeated, to find out its exact role. Some experiments should also be done to show whether in such a mixture the nitrogenous materials in potato convey any special advantage; Chick (80) has had some remarkable results with them, but their importance has never been fully investigated.

d. Cottonseed Meal

Cottonseed, freed from the vestiges of cotton left after ginning, and then decorticated, can be made to yield not only the oil that is its chief commercial asset but a high-protein cake, normally used for stock feeding. There is no obvious reason why the cake, like other oilseed products made in similar ways, should be used only for the feeding of animals. A small part of the United States production is made into a

flour for human consumption, but it is used chiefly for certain special qualities needed in baking, and not for its protein. In Uganda it has been incorporated in a few preliminary experiments in the diets of children, but this work is not yet far enough advanced for any conclusions to be drawn. There are various disadvantages. It is necessary to ensure that the gossypol present is harmless, and that the cottonseed allergens are unlikely to give rise to reactions. The color of the flour is also intensely yellow, and everything with which it is mixed turns yellow or brown. None of these disadvantages is likely to be insuperable, but they must be circumvented if the cottonseed protein is to be used extensively. (See also discussion of cottonseed flour in Chapter 17.)

III. OTHER FORMS OF PLANT FOOD

1. Plankton

The total photosynthetic activity of the sea has been estimated to be ten times that of the land, and the greater part of plant life grown in the sea is made up of the various kinds of plankton (unattached, floating algae; see Chapter 30). The most usual chain of nutritional events is that the phytoplankton is eaten by the zooplankton, which is eaten by crustaceans, which are eaten by small fish, which are eaten by larger ones. The process is subject to the usual factor of partial utilization, and it has been calculated that 1 kg. of a large fish such as a cod represents 100,000 kg. of plants grown in the sea. The blue and fin whales, which are about 6 meters long at birth, reach a length of about 20 meters and sexual maturity in about 2 years on a plankton diet, but some recent calculations suggest that this achievement is an outstanding monument to persistence and travel (81). Man finds it difficult to compete if he wants to make a similar direct use of the same diet.

In 1891, Herdman (82) gave what he believed to be the first account of the use of plankton as human food; proposals without trial had, it seems, been under discussion by biologists for some time, especially by the Prince of Monaco. On the night of July 12th, Herdman was with seven sporting companions on a steam yacht near the entrance to Lyngen Fjord, off the North Cape of Norway. Watching the midnight sun they decided to catch some copepoda for the pot. They put out a small townet, 3½ feet long, and with a mouth 1 foot in diameter, and between 11.40 P.M. and midnight trawled about a mile and a half of sea and caught three large tablespoonfuls of a large red copepod (thought to be *Calanus finmarchicus*). "We conveyed our material at once to the galley, washed it in a fine colander, boiled it for a few minutes with butter, salt and pepper, poured it into a dish, covered it with a thin layer of melted butter, set it in ice to cool and stiffen, had it this morning on thin bread-and-butter, and found it most excellent. The taste is less pronounced than that of shrimps, and has more the flavour of lobster." The dish was shared

by eight people, but "would probably have formed, with biscuits or bread, a nourishing meal for one person." Prompt and sanguine, Herdman wrote his letter to *Nature* on the following day, July 13th. He survived at least until October 6th, when he again wrote to *Nature*, but this time about a striking pink organism.

The German State Biological Institute, at Heliogoland, was said to be investigating the possibilities of using plankton as food before World War II, and in 1941 the British Government was asked in Parliament to consider the appointment of a committee to study its use. Hardy (81), in discussing the question in the light of Britain's hazardous food position at the time, pointed out that one of the great difficulties was the amount of water that had to be dragged before a considerable quantity of plankton could be obtained. He based his calculations on the assumption that 750 g. of dry matter would be required to feed one man for one day. The distribution of plankton was so sparse that it would be necessary to traverse 7500 cubic meters of sea water to obtain this amount, an operation that would take $2\frac{1}{2}$ hours of fast and efficient netting and would obviously be hopelessly uneconomic if it had to be done by boat. He mentioned a proposal to use the fall of tidal waters between islands or in an estuary, and described a small net which was suitable; ten such nets, in a two-knot tideway, might be expected to obtain 267 kg. dry weight of plankton in a day, provided they followed the diurnal vertical migrations of the organisms. The dry substance of copepod plankton has 59% protein, 7% fat, and 20% carbohydrate (83). The 750 g. of dry matter intended for one man's ration would therefore provide 440 g. of protein, 52 g. of fat, 150 g. of carbohydrate, and 2850 Calories: the 267 kg. would provide 148 kg. of protein, 18.5 kg. of fat, 53 kg. of carbohydrate, and 340 times the 2850 Calories. These calculations are somewhat hypothetical, perhaps the most obvious criticism being that 440 g. of protein is about four times the normal intake of an adult man, even in well-fed communities, and probably five or six times the necessary intake. If we take the more realistic view that 20 g. of delicately lobster-flavored protein might be the amount desired or desirable to supplement a diet based on terrestrial plant life, the day's catch of the ten nets becomes the protein supplement (and the addition of 110 Calories) to the diets of 7500 persons.

2. Algae

In China and Japan, seaweeds are used fairly extensively as human food, but although they may contain a high proportion of protein (see Tables II to V, Chapter 30), they seem to be regarded as seasoning rather than as a source of protein. In Japan, the most popular is *Porphyra tenera*, which is harvested in quantities that supply 3000 tons of dry matter each year.* As with other foods, of which dried yeast is a good example, taste is the factor limiting the employment of the algae for the sake of their protein. In some experiments by Morimura and Tamiya (84), a powder of the dried cells of *Chlorella*

* H. Tamiya, Tokugawa Institute for Biological Research, Tokyo, personal communication.

ellipsoidea that had been grown artificially was incorporated in various foods; its taste and appearance were not unlike those of the kind of powdered green tea which is drunk as a suspension, not an infusion, and is somewhat bittersweet in flavor. Perhaps the most valuable finding was that amounts of the powder sufficient to raise the protein content of bread by 20% could be added without affecting the taste adversely. This was achieved with about 6.5 parts of powder to 100 parts of wheat flour, a proportion similar to that used in the enrichment with skimmed milk solids of wheat flour for bread-making in the United States. The powder contained 42% protein and significant quantities of vitamins. As with food yeast, it is remarkable that no intensive research has been done to find out how the taste could be reduced in pungency.

From time to time, the culture of algae for human food has been seriously suggested. In 1946 in Germany, various algae were being used for the small-scale production of fat, and if the food shortage had continued, plans for large-scale manufacture would probably have been put into action, although extensive tests on human subjects had not been carried out. Some algae, such as *Chlorella*, can be made to produce either fat or protein in large quantities, by varying the conditions of culture, and the protein gain could in theory be made of economic importance. There are, however, some important limitations to production. *Chlorella* will divide, under favorable conditions, every 12 hours, and a yield of between 30 and 40 tons of dry matter with 15 to 20 tons of protein could be obtained yearly from each acre of cultivation (about 0.75 ton of dried soybeans with 0.25 ton of protein can be obtained from an acre in a year). Production of this intensity could not be achieved in a "natural" site such as an open pond; a suitable medium for growth would be needed, the cost of harvesting the dilute culture would be formidable, and it would be impossible to prevent contamination (85). A closed system would presumably be necessary, and that would be expensive. To achieve maximum growth, the organism needs a medium containing 5% carbon dioxide, and not only must the medium be replenished as its constituents are used up, but some of the products of metabolism must be removed before they achieve high concentrations. Harvesting would still be difficult, and there would remain the need for careful drying, always costly and often hazardous to the quality of protein concentrates (86). It is possible that experience in the growing of antibiotics, and the development of the methods for continuous bacterial growth that are becoming practicable for various commercial purposes, will show how the cultivation of algae can be made profitable; for the moment, the cultivation does not appear to

offer great hopes for the increase of the world's protein supplies, and it seems better to rely on higher plants. (See also discussion of this problem in Chapter 30.)

3. Food Yeast

The history of the attempts to use food yeast for human consumption has not so far been encouraging. An important stage in pilot plant development was reached in Britain about 1940, in the Chemical Research Laboratory of the Department of Scientific and Industrial Research, and a full-scale plant using the knowledge gained was erected in Jamaica. The raw material used there was waste from the processing of sugar cane, but sulfites can also be employed, and were in Germany during the 1939–1945 war, for production reaching 39,000 tons of dried yeast yearly. Sulfites from paper mill wastes are being used in the United States, where two large factories are in operation, with a combined capacity of about 4000 tons yearly (87).

The organism used in these ventures has been *Torula utilis*. It can utilize five-carbon sugars that are not available to the *Saccharomyces* yeasts used in baking and brewing, and as a dried powder it is light in color and not unpleasant in taste. The powder has about 50% protein, and, as the protein is rich in lysine, it should be a good supplement to cereal proteins. Sure (88) and others have demonstrated its supplementary value for wheat and corn meals in animal-feeding experiments, and at one time, in all hospitals and government institutions in Malaya, about 7 g. of dried yeast was added to the diets each day (30). Various experiments (89) were made in Britain to see how much yeast could be incorporated in a day's food. Biscuits and breads were baked with it, and it was tried in soups and sausages. Unfortunately, the amount needed to spoil an acceptable flavor is small, and it is difficult to persuade most people to take more than about 10 g. in any form in a day. It has also been found that larger amounts may cause diarrhoea. Nevertheless, the recent increase in production in the United States and the increasing incorporation of yeast in various foods for human use—about one-fifth of the present production is going into sausage mixes and soup powders—suggest that it may yet attain importance for its protein content. Up to now, it has really been of value only as a source of the B vitamins.

The Jamaica factory was closed down because it could not find a large enough market for its product. It is easy to point out that it would have been worth while discovering, before the plant was built, the extent of sales that might be expected, but it is also clear that research is urgently needed to find means of modifying or extracting entirely

the disagreeable taste and the substances responsible for the intolerance. (See also Chapter 29.)

4. Leaf Proteins

Protein synthesis is one of the chief activities of the leaf, and leaf proteins are comparable to animal proteins in their amino acid composition (90). The young leaf is especially rich in protein, and it is common agricultural practice to take advantage of the richness for the making of fodder. In some plants, of which wheat and potatoes are examples, the protein that is harvested from the mature crop is approximately the same in amount as that in the leaves when they are at their maximum in weight and protein content; at the time of the harvest the leaves may have lost most of their protein, but much of it has gone to the seed or storage organ that is the part considered suitable for human consumption, and it is obviously best to wait until it has got there. In other plants, however, much of the protein remains in the leaves, and it would be a great advantage if the leaves could be eaten in large quantities. There are good reasons why that is usually impossible, and although leaves form part of human diets everywhere—in West Africa alone the leaves of at least 150 different plants are eaten (91)—they are mostly of importance for their flavor, or as a source of minerals or vitamins, rather than protein.

The commonest way to use leaves is to throw them fresh into the cooking pot with the staple, but sometimes the leaves are dried and preserved, either as a loose bundle or as a powder. Samples of the powder were obtained by Williams* in Tanganyika and were said to consist of dried leaves from about 30 plants; one sample had been pressed into a flat cake, and this, and the powder itself, contained about 25% protein. Levy *et al.* (92) writing of South African Bantu, also mentioned the preservation of leaves, either sun-dried or cooked in water, and then pressed into cakes that were dried. In either case, the dried material was cooked in water before being eaten. Unfortunately, no information was available about the quantities that were normally eaten, or that could be eaten. It would be of interest to know if they made an appreciable addition to the total protein of the diet.

The great disadvantage of leaf is that it is always associated with large amounts of fibrous material, and separation is difficult. It is obviously necessary to start with leaves containing the largest possible proportion of protein, and it has been suggested that the ideal material would be those grasses that can be grown with 40 to 45% protein. Such a yield, however, can be achieved only under very special conditions, and a more practicable approach is to use young vigorously growing

* C. D. Williams, personal communication.

leaves that have less protein but are more easily available. (See also Chapters 3 and 25.)

There are many ways in which a juice can be obtained from leaves—by pressing them intact, by destroying the osmotic control of the cells, or even by applying a stream of compressed air to the leaf stem—but the juice so obtained is almost protein-free. The proteins are mostly in the plastids, and a combination of rubbing and beating is necessary to free them. The grinding teeth of the ruminant and the ordinary domestic mincing machine appear to be two of the more efficient mechanisms for the purpose. Great difficulties are met when attempts are made to devise machinery that will deal satisfactorily with large quantities of leaves of different kinds, but a suitable mill with a combination of propelling and beater arms has been described (93, 94). The protein-rich juice having been obtained, it is necessary to consider how the protein can be extracted. It can be precipitated with acid, but the precipitate does not settle out easily and is difficult to separate from the rest of the juice. It is better to use heat coagulation, but an ordinary heater element rapidly acquires a thick layer of the coagulum that is an efficient heat insulator, and live steam is much to be preferred although it does not always give the greatest possible yield. Most of the water can be removed from the curd by filtering or centrifuging, followed by pressing, and the final product has about 50% protein.

Other methods are possible. Sullivan (95) extracted dried grass with sodium hydroxide and precipitated the protein by making the extract acid. The yield was good, but drying is expensive, and becomes more so with increasing richness of the leaves in protein, because it is usually found that the more protein is present, the more water is held by the leaf. Of the other methods, one that seems to deserve further investigation is digestion by *Clostridium roseum*, originally used for the isolation of rubber from *cryptostega* leaves, but capable of being applied to leaves of other plants (96). As Pirie (94) has suggested, the process might be well suited to recover protein from the fibrous residue left after mechanical separation; the protein is otherwise extremely difficult to free.

There are clearly a number of obstacles preventing the immediate use of leaf protein for human food. The only sample that has been offered to the present writer was unpleasant in taste and deep green in color, and it seemed unlikely that such a product would find ready acceptance. The harvesting of leaves in such a way that the protein value of the rest of the plant is unharmed is also important; it may be advisable to select plants for their leaf yield, and leaves in which the

conditions for protein synthesis are optimal. According to Pirie (94), "The efficient plant will probably have thin leaves growing all the way up a succulent stem and forming a continuous mass of several feet thick. This will ensure that little light reaches the ground where it is wasted, that as large a proportion of it as possible is stopped by photosynthetic rather than simply opaque structures, and that much of the leaf area is in that part of the total volume of the crop that is sheltered from full sunlight." In temperate climates shade-grown leaves have a higher protein content than well-lit leaves under identical conditions, and if the same holds for warm climates it might be profitable to use the lower regions of moist tropical forests for deliberate leaf culture. There are also obvious possibilities in such abundant and little-used material as the leaves of sugarcane, cassava, and bananas.

IV. FUTURE EXTENSIONS OF THE USE OF PLANT PROTEINS

1. The Theoretical Basis of Selection

Dietary regimes that are founded on cereals are likely to contain much more protein than those founded on cassava, plantains, or sweet potatoes: for every 100 Calories, 15 are protein Calories in wheat, 11 to 12 in corn, millet, or sorghum, 8 in rice, but only 2 in cassava, 4 in plantains, and 6 in sweet potatoes. It is obvious that whole diets, and not merely their staples, must be considered when values are being compared, but the protein Calories of the staple usually serve as a rough guide, in practice, to the amount of supplementation needed.

The question of quality of protein is secondary in importance to amount of total protein. So far, no disease of man is known that can be attributed to an amino acid deficiency, but there seems to be little doubt that in many parts of the world young children, and possibly some adults, suffer from protein deficiency. Quality of protein has largely been determined by experiments on small animals, and the extremely rapid rate of growth of those animals has the very useful function of exaggerating amino acid requirements. It seems probable that for man, and even for the child when he is growing fast, proteins of less than the highest theoretical biological value are adequate, provided that there are enough of them.

There are very few generalizations applicable to quality of plant protein, and perhaps the only one of importance is that cereals are not rich sources of lysine. In theory, therefore, plant materials rich in lysine, such as yeast, maize or wheat germ, or soybeans, should be added for good balance. Of these, soya can be used provided that it is properly cooked, but the germs share with yeast the property of being

inclined to cause digestive upset if they are given in amounts that appreciably raise the protein intake. Mixtures of soya and wheat, barley, and corn, with the cereals malted, were used in the German feeding trials already referred to. Theoretically, the best of the mixtures, despite the presence of the soya, was deficient in lysine, and also deficient in tryptophan, cystine, leucine, isoleucine, and valine, when compared with human milk protein (the reference protein in the experiments), but the children ate enough of the diets to ensure an apparently adequate intake of all the essential amino acids. At some time in the future it may become necessary to insist on the consumption of minimal amounts of protein, and then quality may possibly have to be considered in detail. Until that time, quantity will be more important.

Ideally, proteins that are being introduced to improve diets should not need difficult preparation. Some of the beans that can be cooked simply after being dried seem to be most worth encouragement, but there might be great advantages to be gained by the selection of cereals for their protein content, as apart from the usual basis of weight of yield. Protein can be increased, in most cereals, by increasing the amount of nitrogen available in the soil they are growing in, but nitrogenous fertilizers nearly always involve the expenditure of fuel or energy for their production, and their price can easily become intolerable. It may be necessary to pay the price; the diversion to food of proportions of income that would at present be considered fantastic may become the price of survival, and it is in these circumstances that the bizarre forms of plant proteins—algae, leaf proteins, and so on—may have to be exploited. To some people, limitation of population may seem a more completely satisfactory solution.

Assuming concentration on crops at present available, a plea may be advanced for the best use of the protein they contain. Where possible, the whole rather than a part selected for purity of color or texture should be employed, and a metabolic unit such as the germ of a cereal should be milled into the rest of the grain. Unfortunately, it is necessary to test exhaustively the effects of any alteration in the technique of processing. To take well-known examples, undermilling may reduce nitrogen absorption, and the retention of fat may impair keeping qualities. Heat, which usually improves at least to the extent of making a food edible, may be capable of more than that if it is judiciously used; excessive heat, on the other hand, may be harmful. Heating in the presence of carbohydrate may reduce protein values greatly by destroying, or making unavailable, some of the amino acids, and the same processes may go on at ordinary temperatures if certain conditions, such

as the presence of the requisite amount of moisture, are fulfilled. The alterations in quality of protein during storage have not been fully explored, and it may be found that, under proper control, improvement and not deterioration can be ensured. The germination of soybeans and the malting process are examples of the principle, and other possibilities, perhaps making use of exogenous as well as endogenous enzymes, are not difficult to envisage. Unless some way of using nitrogen-fixing bacteria can be devised, the procedures are unlikely to add protein, but they might make the most of what is there already. (See also discussion in Chapter 11.)

Finally, it is of importance to bear in mind that problems of protein nutrition are largely problems of infancy and childhood. The pig-keeper knows that he need concern himself with protein in the feed only until the young pig reaches a weight of about 100 pounds; and the pediatrician knows that healthy children growing perfectly need less and less protein per unit of their body weight as they grow older. The concentration on quantity that has been stressed, and that on quality if the necessity is ever proved, should always be undertaken with children in mind, and anyone proposing a new food must therefore take into account some of the special features of childhood such as the limited capacity of the stomach and the immaturity of the digestive system.

2. The Assessment of the Value of Foods Intended for Human Consumption

The basis of selection of foods for human use is largely empirical. The selection has not yet been approached scientifically. It seems obvious that body composition must bear some relation to food intake; it is known that the composition of body fat is influenced by the fat in the diet, but nothing is known for certain about the relation between dietary protein and body proteins. Very few human bodies have been analyzed, and none of these in full, and present estimates of amino acid composition are nearly all analogies from animal work raised to the power of faith. It is curious that although much is known about the constituents of the individual liver cells in disease believed to be due to protein deficiency (97) there are no complete data for the amino acid content of the whole body of a healthy child, or even for his whole liver; and the data, if they were obtained, would need to be supplemented by similar data for various states of ill health, congenital and acquired, infective and endocrine in origin, and also for the long- and short-term effects of various diets. The plant varies in composition with the nutrition it receives, even to details of its amino acids (98), and so may man for all we know.

In the absence of the information that is obviously necessary, it has been the practice to select proteins for their ability to give the greatest gain in weight of experimental animals, and one method relates the gain to a unit of dietary protein. The chemical analysis of the most successful protein then provides the standard for excellent composition, and egg protein is usually accepted for that purpose. Presumably, the body composition of the animal fed on egg protein must also be accepted as ideal, and analysis of the animal is one method that has been used to measure protein quality. It is strange that cannibalism does not, apparently, triumph over oophagy: Cuthbertson* fed well-homogenized mice to other mice, but found that his stock diet gave better growth. The method can also be applied to a single organ, preferably one very active metabolically, such as the liver (99), and it then becomes within the reach of the physiologist dealing with human beings, because liver biopsy is now a standard procedure that is almost devoid of risk.

The ability of a protein to provide for the synthesis of other proteins, especially of serum albumin and other substances that are made in the liver, has been adopted in clinical work with malnourished children in Kampala as a measure of that protein's efficiency. Liver biopsy is not necessary, because the substances are discharged into the serum, and the serum can be obtained by venous puncture in the usual way. The method is being used to compare various plant proteins with milk protein, that having been found to be most certain to provide clinical improvement and rapid synthesis of nitrogen-containing substances. The children who are being investigated are ill and highly abnormal, and it is therefore quite legitimate to argue that the results are not the same as would be obtained with completely healthy children. The reverse kind of experiment would be possible in which healthy children were given test proteins until they showed signs of impaired synthesis, but such experiments would be difficult to justify.

It is of course possible to observe, in trials lasting many months, the effects on growth and development that are produced by dietary regimes in which various proteins are included. There are many difficulties—the appetites of children cannot be forced; most ailments reduce appetite drastically; appetite is strictly individual, and no two children of the same age eat the same amounts; metabolic requirements differ by the amounts needed for the small movements that are independent of easily measurable bodily activity; effects depend on storage, on the presence and absence of mineral and vitamins; and so on—but there is no doubt that however perfect the chemical analysis and the animal work may be, the final answer giving the value of a food for a human

* D. P. Cuthbertson, Rowett Research Institute, Aberdeen, personal communication.

being can be obtained only from human beings. In theory, it can be obtained from an adult; in practice, the child, whose compelling need for growth must be satisfied, is the better subject.

It is extremely important that all new foods, and even old foods prepared in new ways, should be thoroughly tested before they are allowed in general use. After the chemical and animal tests have determined the theoretical suitability, the lack of toxicity, and the biological value, trials must be made with adults, older, and finally younger children. The whole progress must be repeated if changes are made in the formula or the method of preparation.

3. Practical Measures for the Future

If this chapter has served its purpose, it should have indicated the lines on which work for the future on protein utilization should move. Perhaps the most urgent need is for the development of cheap high-protein foods suitable for children of all ages, even the youngest. The general principles of such a food have often been set out: it should be cheap and yet of the maximum quality, bland, capable of being mixed with the rest of the diet, and preferably acceptable because it is a known item of diet even if it is prepared in an unusual way. It could probably not be made cheaply enough unless the raw material was locally grown, and in the tropics, where it is most needed, it could not be distributed satisfactorily unless it was in a dry form. It is not yet possible to satisfy all these requirements. The nearest that has so far been achieved appears to be the Nigerian production of groundnut flour that has been mentioned. A food of the kind that has been prepared in the United States for many years and distributed in relief schemes, but with a basis other than soya flour, is under trial in India. (See page 223 for first results of nutrition tests, also the economics of such a mixture.) Work at Kampala and elsewhere, although necessary as preliminary steps toward large-scale production, has not even reached the pilot plant stage. That stage cannot be by-passed; no commercial manufacturer can afford to omit it, and every government, and every organization seriously concerned with large-scale measures for dietary improvement, must accept it as costly but inevitable. Properly viewed, it is an insurance against great loss; apart from considerations of manufacture, mistakes in child feeding are too expensive, especially in the lasting prejudice that they breed.

In underdeveloped areas, the improvement of agricultural practices and the discovery and introduction into general use of the best varieties of food plants for the particular area, with its special conditions of

climate, insolation, and soil, must be one of the first problems to be attacked. If changes are needed, they must be inserted with the maximum of tact and of understanding, or attempted understanding, of prejudice; the violation of tribal habit or of taboos cannot be undertaken lightly. There must be education at all levels, with the teaching almost infinitely adaptable but yet coordinated. The child at school is perhaps the most receptive audience, and schemes such as that supported by the Nuffield Foundation at the Gayaza School for Girls, near Kampala, where a school farm is being run closely linked to a school curriculum centered on the domestic use of the food grown, can be expected to produce the best results. The school child, however, is being educated for the future, and is far from wielding any executive power. The education must therefore embrace all the departments of government, and all grades of the executive. The officers must be made aware that every individual is concerned with the problem, and cannot escape his share in the solution of it. There will soon be a universal problem in nutrition, and especially, it seems, in protein nutrition. The solutions found by individual countries may then be seen to have a value that is much more than parochial.

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CHAPTER 10

VEGETABLE PROTEIN ISOLATES

ALLAN K. SMITH

I. INTRODUCTION

The term protein isolate (or isolated protein) is usually restricted to the designation of a chemically isolated protein; analysis of a protein isolate, after correction for moisture and ash, shows that it is approximately 100% protein. Closely related to the isolates are the "vegetable protein concentrates," a term used broadly to designate vegetable products which are valued because of their high protein content. Some of the concentrates are specially processed and mechanically refined to increase their protein content for food and industrial uses, and some of them containing 50 to 90% protein are the immediate raw materials for the isolate. Indeed in some applications they may be considered as competitors.

The isolation of vegetable proteins is a comparatively new industry, started in Chicago in 1935 to produce an isolate from soybeans for the paper industry. Zein, the protein isolated from corn, became a commercial product about three years later. Isolated peanut protein is produced only in England. The production of protein isolates from other sources has been investigated, but none is yet in commercial production. Wheat gluten will range in protein content from 80 to 90%, and in this chapter it is considered as a protein concentrate; when purified to contain above 90% protein it is called gluten protein.

The purpose of this chapter is to present a general discussion of commercial vegetable protein isolates, their source materials, methods of preparation, and uses. This general discussion is followed by specific examples of production, properties, and utilization of certain of the protein isolates.

II. SOURCE MATERIALS FOR PROTEIN ISOLATES

1. Oilseeds

The oilseeds which are processed commercially for oils and meals are described in the chapters of Part II. Table I gives their approximate

analyses and their percentage of hull or seed coat. Some of these oilseeds have already been used for the production of protein isolates or have been investigated for this purpose; preparation and properties of isolates prepared from these commodities will be described later in this chapter. Since more experience is available on the preparation, properties, and uses of isolates from soybean, its isolate is reported in much

TABLE I
APPROXIMATE COMPOSITION OF OILSEEDS^a

Oilseed	Crude protein (N × 6.25) (%)	Oil (%)	Ash (%)	Hulls (%)	Oil in dehulled seed ^b (%)	Crude protein in dehulled and defatted seed ^b (%)
Soybeans	43	20	5.0	8	21.8	52
Cottonseed ^c	21.5	21.6	4.2	36	—	—
Cottonseed kernels	32.5	36.4	4.7	—	—	63
Flaxseed	27.0	42.0	3.4	41	—	—
Flax, dehulled	30.3	55.7	3.5	—	—	65
Peanuts	—	—	—	20–30	—	—
Peanut kernels	30.3	50.0	3.0	2–3.5 ^d	—	57
Castor	18.9	49.5	2.8	25–30	66.0	69
Sunflower seed	19.5	29.3	3.4	43	49.8	61
Safflower	13.5	32.8	3.2	49	62.6	63

^a Moisture-free basis. (See also data on these seeds in Part II.)
^b These data will vary considerably with the efficiency of dehulling, oil removal, variety of seed, and climatic conditions of growth.
^c Acid delinted.
^d Red skins or testa.

greater detail than the others. Specific application of soybean protein for food use is described in Chapter 15.

2. Cereal Grains

Among the cereal grains, the proteins of corn and wheat have been studied most extensively. In the United States, the wet processing of approximately 135 million bushels of corn produces nearly 400,000 tons of corn gluten containing approximately 60% protein. Only a small percentage of the total gluten is processed for the alcohol-soluble protein zein. Both the wet and dry corn millers separate corn germ which, after removal of the oil, contains about 25 to 30% protein. This germ fraction, comprising about 11.5% of the corn kernel, has never been studied seriously as a source of protein isolate. It does not contain any zein. Small quantities of wheat gluten separated from wheat flour are

used primarily in the manufacture of monosodium glutamate and for the enrichment of bread and other cereal products.

III. PROCESSING MEAL FOR PROTEIN ISOLATION

Steam treatment of solvent-extracted oilseed meals, for the purpose of destroying the antinutritional factors they contain, is an important step in producing feed from soybeans and some other oilseed meals. Such treatment, although improving the nutritional value of the meal, denatures the protein and makes the meal unsuitable as a source of protein isolate. Oilseed meals processed in screw- and hydraulic presses are likewise excluded as materials for protein isolation, as these methods seriously discolor as well as denature the protein. Thus oilseed meals for protein isolation are limited to solvent-processed meals from which the solvent has been removed with only a moderate denaturation of the protein.

The rate of protein denaturation (1-3) is slow when the moisture content of the meal is low and the temperature is moderate, but at about 80° and above, with normal or higher moisture content, denaturation is rapid. The extent of denaturation may be established for soybean meal by determining the solubility of the nitrogenous compounds in water (4) or by measuring its urease activity (5), and, for cottonseed meal, by measuring its nitrogen solubility in 0.02 *N* alkali solution (6).

The solubility measurements for soybean meal are made by shaking 2.5 g. of finely ground meal in 100 ml. of water for 30 minutes, removing the insoluble residue in a centrifuge, and determining the per cent of dissolved nitrogen compounds in the supernatant. The factors affecting the dispersion of nitrogenous compounds in water, such as mechanical agitation and temperature of extraction, have been described by Smith *et al.* (4).

Figure 1 shows the results of experiments by Belter and Smith (7) on the dispersibility of undenatured soybean meal in water (*pH* 6.6) and the change in dispersibility which occurs with steam treatment in an autoclave at atmospheric pressure. The rate of change in solubility in commercial processing equipment would probably be different from that for the laboratory experiment; however, these data can serve as a guide in establishing specifications for procuring soybean meal for making isolate. The factors which affect protein solubility in soybean meal likewise have corresponding effects on the solubility of protein in other vegetable meals.

The unit operations in commercial oil extraction of soybeans with low-boiling hydrocarbon solvents are (1) cracking the beans, (2) dehulling, (3) flaking, (4) solvent extraction, (5) desolventizing, and (6) toasting or steaming. Belter and Smith (7) have shown that little de-

naturation of the protein occurs in the first four operations; in most plants some denaturation occurs in the desolventizer, but the greatest change is in toasting for improvement of the nutritional value of the meal. Table II shows the solubility in water of the nitrogenous compounds of soybean oil meal after each of the processing steps and points

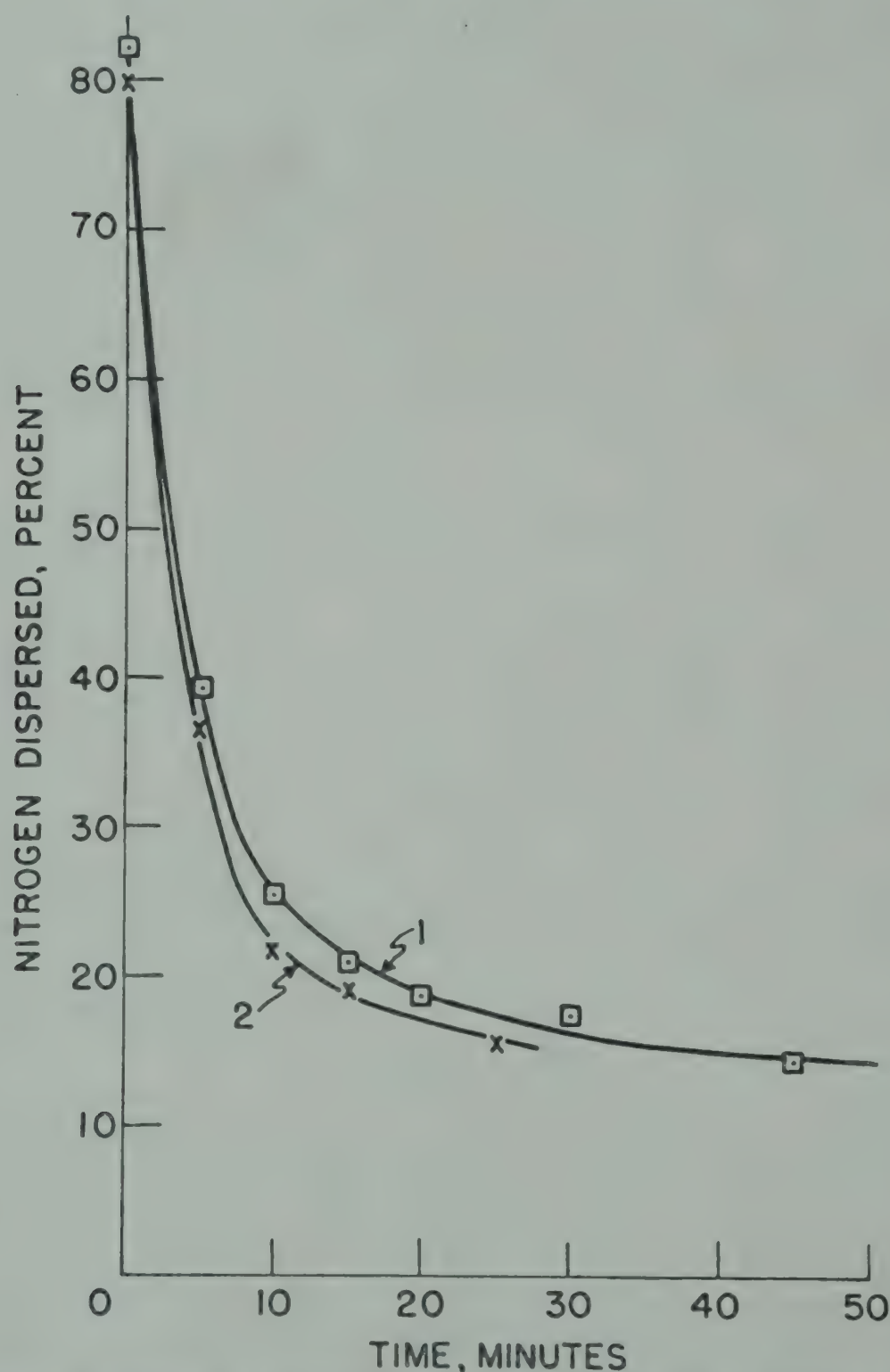


FIG. 1. Change in nitrogen dispersibility of soybean meal in water with increasing time of steam treatment of the meal. Curve 1, the flakes steamed after solvent extraction of the oil; curve 2, the flakes steamed before solvent extraction. Atmospheric pressure. (Reproduced through the courtesy of the *J. Am. Oil Chemists' Soc.*)

up the need for selecting specially desolventized and untoasted meals for preparing protein isolate.

Certain types of desolventizers, called "vapor-phase" desolventizers, are better than others for preparing undenatured meals. One of these (8, 9) is constructed so as to pass the solvent-wet flakes countercur-

TABLE II
EFFECT OF COMMERCIAL PROCESSING OF SOYBEANS BY SOLVENT EXTRACTION ON
PROTEIN DENATURATION AS MEASURED BY NITROGEN DISPERSIBILITY
IN WATER AT pH 6.6^a

Sample	Plant No.				
	1	2	3	4	5 ^b
	Dispersible nitrogen in per cent				
Whole beans	84.6	81.4	87.9	87.0 84.5 ^c	82.9
Dehuller discharge	84.9	79.3	87.1	84.1	79.3
Conditioning discharge	84.9	70.7	79.5	82.2	80.7
Flaking roll discharge	84.0	78.6	74.2	80.7	77.9
Extractor discharge	87.9	78.4	—	79.9	81.1
Desolventizer discharge	85.3	—	—	—	80.8
Deodorizer discharge	43.7	46.6	48.0	51.6	65.4
Toaster discharge	7.2	—	14.0	39.7	8.2

^a Taken from P. A. Belter and A. K. Smith, *J. Am. Oil Chemists' Soc.* **29**, 170 (1952).

^b No dehulling was practiced at this plant.

^c After preheating to reduce moisture in beans.

rently through superheated solvent vapors and thus avoid the use of steam; soybean meal can be produced in this equipment with 80% or more of water-soluble nitrogen compounds. More recently a flash-type desolventizer, also using superheated solvent vapors as a source of heat, has been described by Belter *et al.* (10); a diagram of this equipment is shown in Fig. 2. It consists essentially of a conveying and desolventiz-

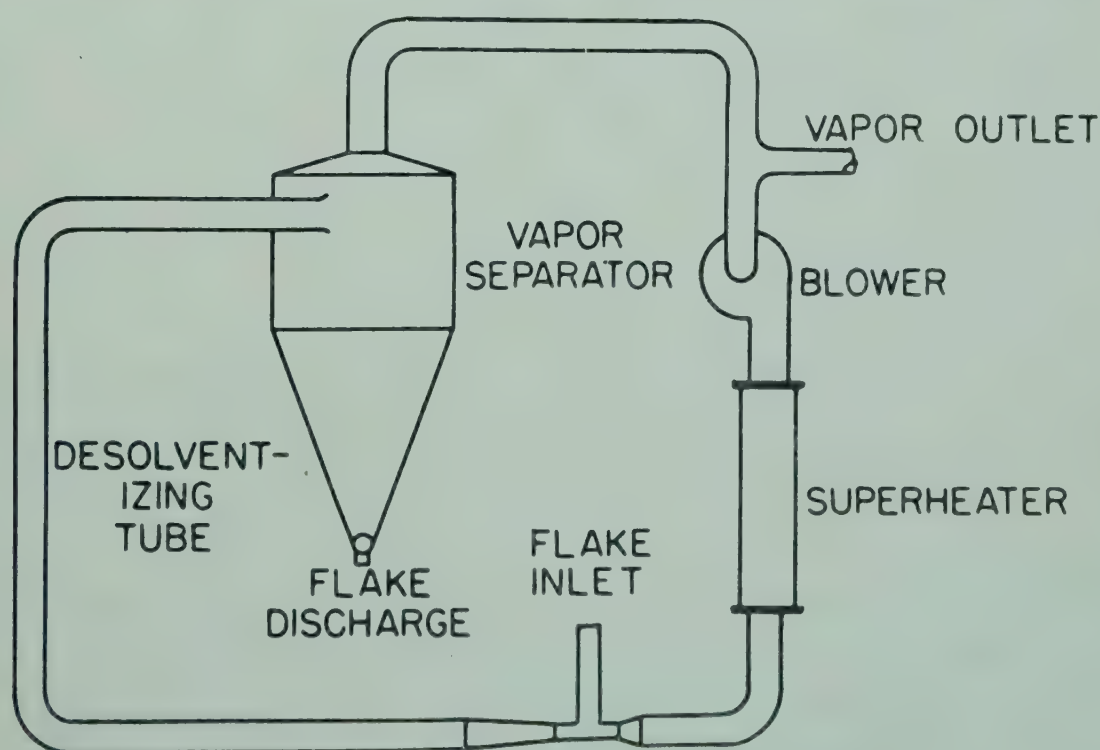


FIG. 2. Diagram of flash desolventizer.

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ing tube into which solvent-saturated flakes are fed and through which superheated solvent vapors simultaneously desolventize and convey the flakes to a cyclone separator. In the cyclone the vapor stream and the desolventized flakes are separated, the flakes are discharged from the system through a barrel valve, and the vapors are recirculated by a blower through the superheater and back into the conveying tube. A quantity of solvent vapors equal to that vaporized from the flakes is continuously withdrawn to a condenser. This type of desolventizer can be used with or without adding steam and has the additional advantage of a retention time of only a few seconds. The flash equipment was originally designed for desolventizing alcohol-extracted soybean flakes but can be used as well with hexane-extracted flakes.

IV. PREPARATION AND PROPERTIES OF ISOLATED PROTEIN

1. Native Proteins

Proteins have been described and some of their chemical and physical properties have been given in Chapters 2 and 3. Native proteins are difficult to isolate in pure form; none of the vegetable proteins has ever been isolated in a truly homogeneous state. The physical structures of natural proteins are particularly sensitive to change by moist heat; in fact the term "protein denaturation" has been defined as "any non-proteolytic modification of the unique structure of native proteins, giving rise to definite changes in chemical, physical, or biological properties" (11). In addition to heat, proteins may be denatured by physical forces such as ultrasonic sound waves, film formation, freezing, irradiation, or pressure, by treatment with organic liquids such as alcohol and acetone, and by action of certain compounds like urea, guanidine, detergents, and enzymes. (See Chapter 5 for a discussion of the effect of heat on proteins.)

2. Commercial Protein Isolates

The methods for isolating proteins on a commercial scale from seed meals are quite different from those used in fundamental studies of proteins; the latter are directed toward isolating specific and homogeneous proteins for physical characterization and for study of their chemical properties. Laboratory methods for isolating specific proteins usually involve dispersion of the protein in neutral salts and precipitation by dilution, by dialysis, by salt dehydration, and sometimes by solution or precipitation with organic solvents. Such procedures require too large amounts of water and chemicals to be suitable for economic commercial operations. The high yields essential for production of commercial iso-

lates from oilseed concentrates are obtained only by extraction of the protein concentrates with an alkaline solution and precipitation with acids. Yield is much more important than protein purity and specificity; a mixture of proteins may be equally as useful as a homogeneous protein. Some denaturation or degradation of the protein may even be an advantage for a particular purpose. Indeed, for industrial application, it is often necessary to modify the protein to provide a particular property such as dispersibility, improved color, foaming capacity, wettability, adhesive strength, viscosity, gel strength, colloid protective properties, or a specific molecular size or shape. Therefore proteins prepared for industrial use are "derived" rather than "native" proteins.

3. Isolated Proteins for Food Uses

An important distinction exists between proteins isolated for foods and those isolated for industrial uses. There are differences in amino acid composition, physical properties, and sanitary conditions in production and marketing. The production of most industrial proteins involves modifications by alkali and other chemical treatments. Their exact nature has not been determined, but it has been observed that there is a loss of hydrogen sulfide and of ammonia, a lowering of protein yield, a decrease in the viscosity of the protein, and the appearance of a humin or other degradation products. These changes signify a loss of sulfur-containing amino acids, and of amino and amide groups, and without doubt result in a change in the balance of essential amino acids from that occurring in the original meal or from that of a protein isolated in an unmodified form. Such conclusions are supported by the erratic nutritional results which have been reported on feeding industrial proteins. Moreover the amino acid composition of industrial proteins may be expected to vary between grades produced by the same processor as well as between different processors; therefore isolated proteins produced for industrial uses are unsuitable for nutritional purposes.

Food and nutritional grades of isolated proteins, particularly soybean proteins, have become available commercially. They are prepared with a minimum of chemical modification and with no apparent loss of amino acids; they should be suitable as a protein source in nutrition. Because of the increasing interest in isolated protein for food, it is important that a clear distinction be maintained between proteins isolated for food and nutritional studies and those prepared for industrial utilization. The potential for isolated protein in food is discussed in Chapter 11, and soybean protein is described in Chapter 15.

V. THE HULL PROBLEM

Seed hulls must be eliminated from oilseed protein concentrates for industrial and food preparations and for producing isolates. They are high in fiber and low in protein, contain dark-colored pigments, and impart unpleasant flavors to food products. Thus, they are undesirable in concentrates used in producing foods, poultry feeds, industrial products, and protein isolates. Table I shows that the approximate percentage of hulls from the various oilseeds ranges from 8% for soybean to 49% for safflower. Their disposal, especially when they are the major component of the seed, as in sunflower, can become a problem in processing oilseeds for specialty uses of the meal. Nelson *et al.* (12) have determined the composition of grain and oilseed hulls, nut shells, and fruit pits, and perhaps their data will be helpful in finding new outlets for surplus hulls. In Canada, sunflower seed hulls are pressed into small logs and sold as fireplace fuel.

VI. USES OF PROTEIN ISOLATES

1. Industrial Applications

A detailed description of the industrial uses of the isolated proteins is given in a later section; in the United States a survey and report have been made (13). Protein isolates were developed originally for their industrial value, with food utilization a secondary consideration.

Protein isolates find industrial applications as sizes, water-emulsion paints, fibers, and for a wide variety of adhesives. They compete with proteins of animal origin and with synthetic polymers of non-protein origin. Vegetable sources of proteins are basically more economical than the animal sources because all animal proteins are ultimately derived from plants. Production of 1 pound of animal protein requires 4 to 10 pounds of vegetable protein. Thus the source materials for the production of industrial animal proteins are limited to the less expensive waste products and temporary surpluses of the meat-packing houses and the dairy and fish industries.

The industrial animal proteins (14, 15) from the packing houses are animal glue; photographic and pharmaceutical gelatin made principally from hides, connective tissues, cartilage, bones, and trimmings from cattle and calves; and blood albumin. The dairy industry supplies casein and dry milk solids; the poultry industry, frozen egg whites and dry egg albumin in both edible and inedible forms; and the fishing industry, a small amount of glue from fish heads and other trimmings.

The total annual production in the packing houses in the United States of edible and inedible gelatin-type proteins is approximately 200 million pounds. Casein production in the United States has declined to less than 8 million pounds per year. Thus it is evident that there are definite limitations to expansion of the supply of animal proteins for industry, and this author believes that any major expansion of isolated protein for industrial purposes must come primarily from vegetable sources.

2. Food Uses

Much has been written about the food requirements of the increasing world population; this subject is discussed in various chapters. (See

Chapters 1 and 9.) The modern use of isolated proteins and protein concentrates for food is in an early stage of development. Progress will depend largely on technological and basic research applied to their promotion. There is always the probability that the isolate or concentrate will be developed to compete with our basic animal proteins, such as meat, cheese, and milk; indeed, this is the subject of Chapter 11. Well-established food habits and customs will make any shift in that direction slow and gradual. Most probably such isolates or concentrates will first be found in prepared foods rather than directly in the home kitchen.

Of the various oilseed concentrates developed for food purposes, soy flour, first prepared in about 1935, has made the most progress. The term soy flour is rather unfortunate, as it suggests that it resembles wheat flour, which it does not; soy powder would be a more appropriate name. Soy flour or powder, although used in bread and other bakery products, cannot replace wheat flour because it does not contain gluten. At the present state of development, 5 or 6% of soy flour can be used in bread without practical loss in color, loaf volume, flavor, or other important physical properties.

The chief function of soy flour is to improve the nutritional quality of the bread by supplementation of the proteins in wheat flour. Recent improvements in the quality and uniformity of soy flour, a better understanding as to its use in bread and bakery products, and the increase in the price of non-fat dry milk solids are factors which have been responsible for a substantial increase in its utilization in recent years. The cost of protein in the form of soy flour is about one-half to one-third that of the isolated protein and thus has a decided advantage over the isolate for many applications. (See also discussion in Chapters 11 and 15.)

Besides the use of vegetable proteins for improved nutrition, there is another area of specialty food products which depends on certain desirable physical and chemical properties of proteins. Examples are the soy whips used as aerating agents in confections, soy flour in doughnuts to control fat absorption, and soy milk for babies allergic to cow's milk.

VII. SELECTED OILSEED PROTEIN ISOLATES

1. Soybean

a. Properties

Considerable literature on the isolation of soybean protein, its properties, and suggested industrial applications appeared long before commercial isolation became a reality.

The first publication on soybean protein was that of Meissl and Böcker (16) in 1883; however, the work of Osborne and Campbell (17) in 1898 has been the most popular literature reference. In 1921 Satow (18) published the results of extensive investigations on the isolation and utilization of soybean protein. Although his investigations were only semiquantitative, they contained many useful suggestions. Markley's (19) publication in 1950 on soybeans and soybean products is the most comprehensive collection of information covering all phases of soybean composition, chemistry, processing for oil and meal, nutrition, and utilization.

The recent literature specifically relating to preparation and properties of the isolate includes that of Smith *et al.* (20) and Nagel *et al.* (21). About 92% of the protein of oil-free soybean meal can be extracted with distilled water at a *pH* of about 6.6. Contrary to the behavior of most vegetable proteins, low concentrations of neutral salts reduce the dispersion of the protein. For example, 0.1 *N* sodium chloride in water lowers the dispersion from 92% to 45%, and 0.0175 *N* calcium or magnesium chloride lowers the dispersion of nitrogen components to 21%. This cation effect is overcome by increasing the concentration of the salt or by raising the *pH* of the system (22). Most industrial sources of water would be too high in salt concentration for extracting protein in good yield, and alkali must be used to overcome the salt effect.

Briggs and Mann (23), Smith *et al.* (24), and Smith and Rackis (25) have made electrophoretic studies of extracts of soybean meal. This work identified a minimum of eight different protein components in the water extract of defatted meal; the major fraction is about 75% of the total protein. Wolf and Briggs (25a) by ultracentrifugal studies on the water-extractable protein have shown the presence of four fractions having *s*₂₀ values of approximately 2, 7, 11, and 15 S and an unresolvable fraction having a value of *s*₂₀, greater than 15 S. No truly homogeneous protein was isolated.

The extent of the dispersion of the nitrogenous constituents of oil-free soybean meal at various *pH* values with several different acids and bases is shown in Fig. 3. To obtain a high yield of protein the meal is extracted by adding water and adjusting to about *pH* 9.0. The insoluble residue is removed in a centrifuge and the protein precipitated with acid in the *pH* range of 4.6 to 4.1. The acid-precipitation curve is almost exactly the reverse of the extraction curve of Fig. 3. Protein isolated by this procedure is a mixture of globulins and glutelins, and in laboratory-scale operation as much as 84% of the total protein of the meal is recovered. Thus, with dehulled, oil-free meal containing 50% protein, the yield will be 42% based on the weight of the meal. In large-scale processing, however, the yields will be much lower, owing partly to the lower water-to-meal ratio which is necessary in commer-

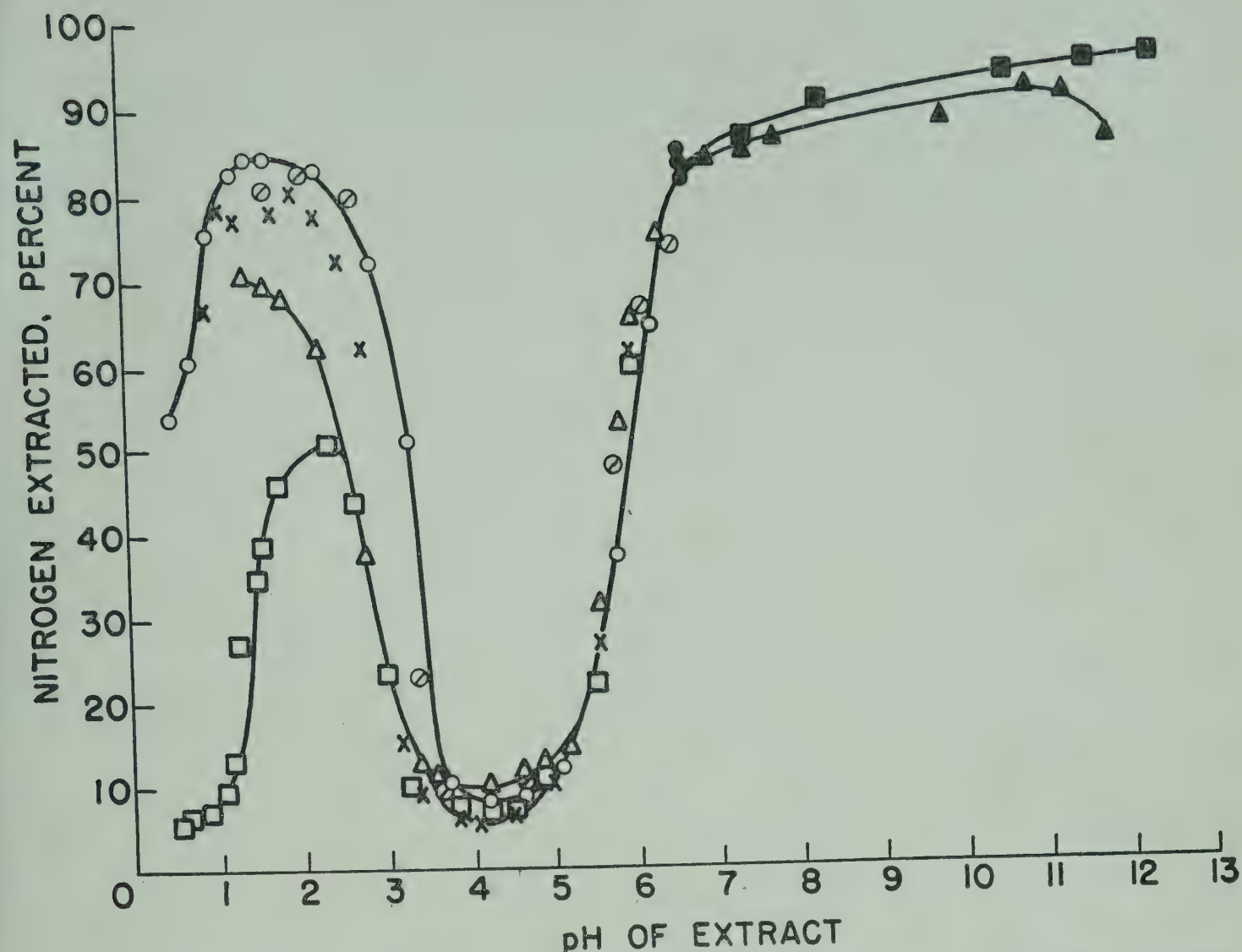


FIG. 3. Percentage of total nitrogen extracted from oil-free soybean meal by various acids and bases. ● Water. ○ Hydrochloric Acid. ■ Sodium Hydroxide. □ Trichloroacetic Acid. ▲ Calcium Hydroxide. △ Sulfuric Acid. ⊙ Phosphoric Acid. × Oxalic Acid. (Reproduced through the courtesy of *Ind. Eng. Chem.*)

cial operations and partly to loss of protein through chemical modification (26, 27) into a more soluble form. A yield of 30% would be considered good.

b. Soybean Whey

The filtrate or centrifugate from the wet protein curd contains albumin, proteoses, peptones, non-protein nitrogen, sugars, trypsin inhibitor, urease, lipoxidase, and additional enzymes and water-soluble components of the bean. This solution, with its many interesting components, is called "soybean whey" (24, 28), and its solids constitute about one-third of the original meal. Although several components of the whey have potential value, economic methods for their recovery have not yet been developed. The whey has a high biological oxygen demand, and its disposal may become a serious problem for some soy-protein processors. It has been estimated that for each ton of protein produced the whey disposal in terms of biological oxygen demand is equal to that required for the sewage disposal of a city of 10,000 people.

c. Commercial Isolation

Belter *et al.* (26) have described pilot-plant operations which demonstrate the equipment and problems of large-scale production; Fig. 4 is a flow diagram of the process. Extraction of the protein from the meal is carried out in unit No. 1 at a water-to-meal ratio of 12:1, with an alkaline solution at about pH 9.0. A second extraction with

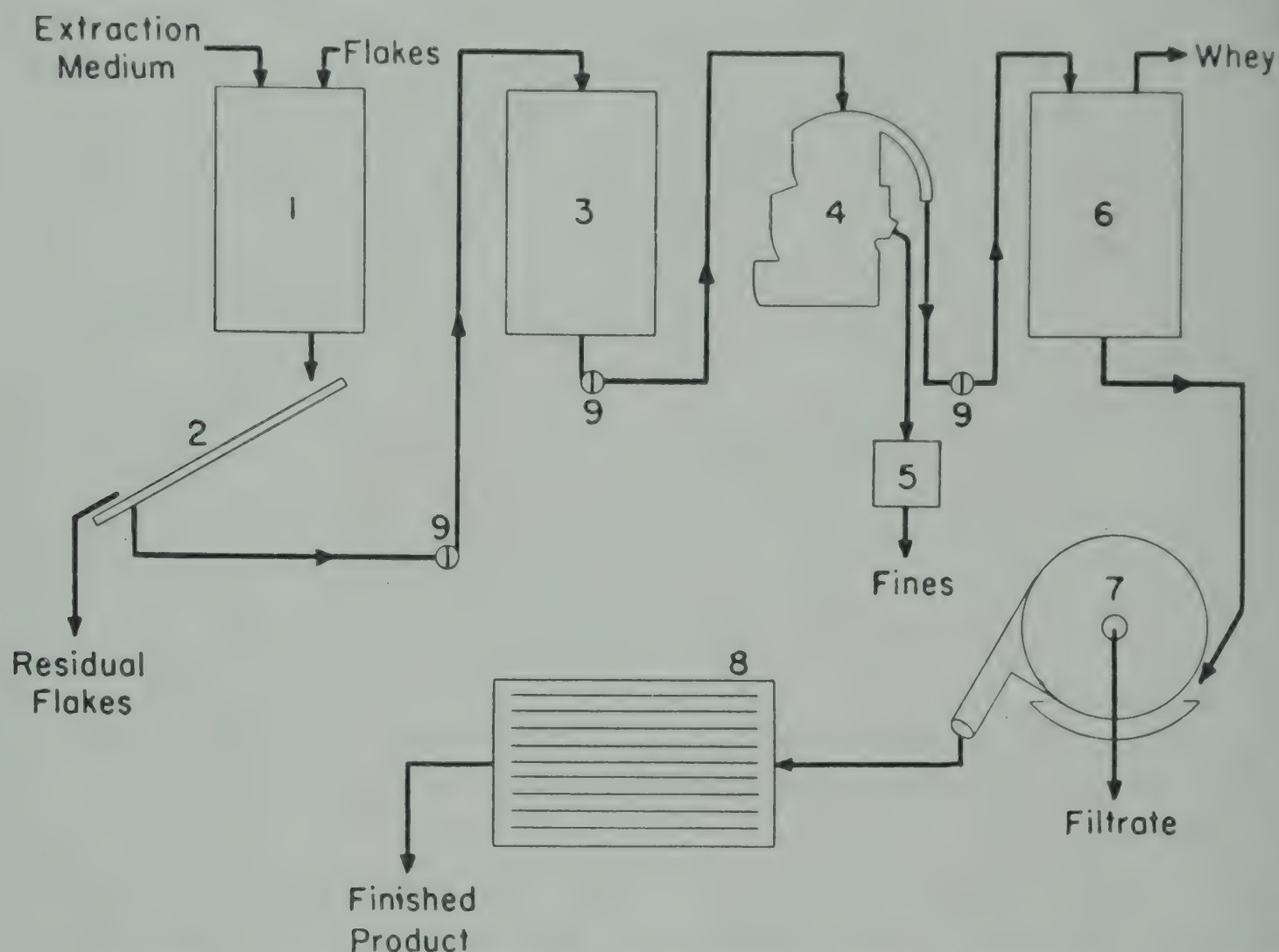


FIG. 4. Flow diagram for pilot-plant production of soybean protein: (1) tank for extracting the protein; (2) gyrating screen (80 mesh) for separating undissolved meal from solution; (3) solution-holding tank; (4) centrifuge for removing "fines" from protein solution; (6) protein precipitation tank; (7) vacuum filter; (8) dryer; and (9) pumps. (Reproduced through the courtesy of *Ind. Eng. Chem.*)

another 5 or 6 parts of water is made and is added to the first extract to increase the yield.

The first difficulty encountered in the process occurs in operation No. 2, that of separating the protein solution from the insoluble residue. This separation cannot be effected by filtration; therefore, the coarse insoluble residue is removed by passing the extract through an 80-mesh screen. The fine particles passing through the screen are removed in a continuous-discharge type of centrifuge at unit No. 4. Unit No. 3 is a storage tank used only for supplying the protein solution to the centrifuge. The protein is then precipitated from the clarified solution in unit

No. 6 by adjusting to pH 4.5; the curd is allowed to settle to the bottom of the tank, and the supernatant solution or soybean whey is discarded. When produced for certain industrial application, the protein curd can be bleached and chemically modified to give a protein with the desired solubility and viscosity properties (29). In these operations the protein is redissolved, reprecipitated, and recovered on a drum-type vacuum filter with a string discharge, unit No. 7. When the protein is being prepared for food and nutritional purposes, chemical modifications which might destroy some of the essential amino acids should be avoided. The protein curd, which is approximately 75% water, must be dried carefully to prevent darkening the color and insolubilizing the protein.

Sodium sulfite may be used in the extraction process when producing industrial-type protein to reduce the activity of microorganisms and to assist in bleaching the protein. Further bleaching is accomplished by precipitation of the protein curd with sulfur dioxide. Other bleaches (27) are sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$), zinc dithionite (ZnS_2O_4), and peroxides. The protein is bleached to a color equivalent in brightness to that of casein.

d. Industrial and Food Uses

According to a survey of the industrial applications of soybean meal, isolated soybean protein, soy flour, zein, corn gluten, and other protein concentrates (13), the principal uses for isolated soybean protein are in pigment coating of paper, wallpaper coating, water and latex paints, lamination of fiberboard and shotgun-shell tubes, insulating board, fire-foam liquid, printing inks, leather finishing, felt-base floor covering, and other formulations for sizing and adhesive applications. Total annual industrial utilization in the United States in 1951 was estimated at 27 million pounds, with pigment coating of paper accounting for more than half of the production.

A large potential industrial use of soybean protein is for textile fibers. Wormell (30) has reviewed the chemistry of the formation of textile fibers from proteins and described their properties. Fibers comparable to the casein fiber, Aralac, which was in commercial production in the United States during World War II, have been made experimentally by the Ford Motor Company (1937), The Drackett Company (1940), and the U. S. Department of Agriculture (1942). The greatest weakness of Aralac was its low wet strength. Likewise the low wet strength of the soybean protein fiber was its poorest characteristic. The British development of a commercial fiber from peanut protein and the American development of a fiber from zein support the belief that a successful fiber may be made from soybean protein. Such a development would be expected to increase greatly soybean protein production.

Several companies are investigating isolated protein for food uses, and reports indicate that a substantial increase in its utilization can be expected. (See Chapter 15.) An enzymatically-(pepsin) treated protein

is now a whipping agent (31, 32) in candies and desserts. A new bland-tasting product called Gelsoy (33), containing about 55% protein, has been incorporated in frankfurters, meat loaf, and canned meats for the prevention of fat and water separation, and in low-fat frozen confections for the control of "overrun." Other suggested uses are as a bread softener (34) and for heat sealing the cork into crown seals. Piper and Morse (35) and Smith (36) have reviewed oriental methods of using soybeans as food. (See also Chapter 9.)

Dehulled soybean meal has several industrial applications which, on a tonnage basis, have exceeded the utilization of the isolated protein. The meal is used for plywood glue, wallpaper coating, and adhesive formulations for the manufacture of paper products. The 1951 survey (13) gave the industrial utilization of the meal in the United States as 51.5 million pounds. The largest single use for the meal, for plywood glue in the Douglas fir plywood industry, amounted in 1951 to 35 million pounds. Recent reports indicate that this has increased to over 100 million pounds, thus showing a decided upward trend. Chang (37) has described a new use for undenatured soybean meal as a sugar liquor defecant in the sugar-refining industry. This application has the advantage that the meal is recovered and sold as a stock feed.

2. Peanut

a. Properties

The peanut has a cellulosic shell which makes up 20 to 30% of its total weight. The seed or kernel contains 45 to 50% oil, 25 to 30% protein, 5 to 12% carbohydrate, 2.5% ash, and about 3% crude fiber (38). Thus, the oil-free meal contains about 50 to 60% crude protein. Hoffpauir (39) has published a review of the literature on peanut composition. (See also Chapter 16.)

The red skin (testa) comprises 2.0 to 3.5% of the peanut kernels and contains tannins and related pigments (40-42) which, unless removed early in processing, seriously darken the color of the protein.

Arthur (43) has reviewed the research prior to 1952 on the isolation, composition, and properties of peanut protein; Baringer (44) has prepared an annotated bibliography of the work of the Southern Regional Research Laboratory on peanuts and peanut protein for the years 1942 to 1953; and Smith (45) has recently reviewed peanut protein utilization in comparison with that of other vegetable protein isolates. Fontaine and Burnett (46) and others (47, 48) have determined the extent of nitrogen dispersion of oil-free peanut meal in solvents of various pH values; their results are remarkably similar to those for soybean meal shown in Fig. 3. It was shown that about 90% of the peanut meal nitrogen can be extracted with water at a pH of

about 6.7, whereas the minimum extraction of about 7.7% occurs at pH 4.3.

b. Utilization

The techniques of solvent extraction have been studied and made available (49). Pilot-plant studies were made to demonstrate equipment and methods for the commercial production of isolated peanut protein (50, 51). Properties (52) and processing variables in peanut protein preparation (53) were reported. Utilization of the protein for plywood glue (54, 55), paper coatings (56), window-shade sizes (57), textile fibers (58, 59), and similar purposes (60-62) has been demonstrated as possibilities. Thus laboratory and pilot-plant researches have demonstrated that peanut protein isolate has properties satisfactory for food and industrial uses.

Commercial development in the United States will depend on the success of research, now in progress, for improving the mechanization of harvesting and of drying the seed (63) in order to reduce the cost of production to a basis more competitive with other oilseeds. The United Kingdom has been more successful in employing peanut protein. It has been reported (64) that the British Extracting Company, Ltd., in Bromborough, England, has a solvent-extraction plant with a daily capacity in excess of 200 tons of kernels per day, and that the Imperial Chemical Industries, Ltd., isolates the protein in a plant of 10 tons daily capacity. The isolate is principally converted into a textile fiber known as Ardil at a plant in Dumfries, Scotland.

3. Cottonseed

a. Properties

Considerable work has been done on cottonseed and cottonseed kernels (meats) toward the development of a protein isolate for commercial application. One of the problems seriously interfering with the success of this development is the presence of dark-colored pigments, principally gossypol and gossypurpurin (65, 66), occurring in the kernel. In the usual methods of processing for oil and meal, part of the pigments react with the protein to form dark-colored reaction products which are difficult, if not impossible, to remove.

The early investigators of cottonseed protein were Osborne and Vorhees (67) and Jones and Csonka (68). In their studies, they used salt, dilute ethyl alcohol, and sodium hydroxide solutions, to fractionate the protein into fractions I and II of high ash content, a pentose protein, a glutelin, and α - and β -globulins. They believed the high ash-yielding fractions to be phospho-

proteins. More recent work by Fontaine *et al.* (69) indicates that the high ash may come from the phytin in the meal or possibly from a combination of phytin and nucleic acids (70).

Olcott and Fontaine (71–73) and others (74) have reported on the composition and dispersion characteristics of defatted cottonseed kernels. On an oil- and moisture-free basis, the meats contain approximately 10% nitrogen or about 63% crude protein. They found many salts which at 1.0 *N* concentration would disperse 80% or more of the nitrogen. In studies on the extent of the dispersion of nitrogen compounds as a function of *pH*, they found it necessary to raise the alkalinity above *pH* 8.5 to attain good dispersion values. At *pH* 9.0 the amount of nitrogen dispersed was markedly affected by the presence of salts. For example, calcium and magnesium salts, in the concentration range of 0.03 to 0.05 *N*, completely suppress dispersion of the globulins. This behavior, it is pointed out, corresponds to the action of salts on soybean protein, as demonstrated by Smith *et al.* (75), except that the cationic effect on soybean proteins was maximum at about *pH* 6.6.

Electrophoretic studies (76) have shown that the solvent used to extract the protein, the type and *pH* of buffers, and the variation of temperature (77) between 0° and 20° had no effect on the relative concentrations or on the mobility of the protein components in the system. These studies showed also that the whole meal contained two major and two minor protein components; the two major components could be obtained in a purified form by dialyzing a salt-solution extract of the meal against selected concentrations of sodium chloride.

b. Utilization

Different methods of extracting the oil and of removing the protein from cottonseed meal have been investigated (78–80) to determine the effect of these modifying treatments on viscosity, yield, and other properties of the protein as they pertain to industrial processing and utilization. Methods for the production of protein fibers (81) and glues (82) have been developed, and the limitation of the isolate in these uses has been demonstrated.

At present there is no commercial production of isolated cottonseed protein. Failure to develop a commercial product cannot be attributed to any single factor, but the difficulty of eliminating pigments appears to be the most serious problem to overcome. If continued research on gossypol should lead to the development of a commercial solvent-extraction process for its removal without excessive denaturation of the protein, then production of a cottonseed protein isolate might be feasible. (See also Chapter 17.)

4. Flaxseed

Table III shows the composition of the hull and embryo fractions of hand-decorticated flaxseed before and after removal of the oil as determined by Smith *et al.* (83). On a full-fat basis the hulls comprise 41.4% of the seed, and on the defatted basis they constitute 60% of the meal. The dehulled and defatted meal contains 10.9% nitrogen or about 65% crude protein. About 97% of the oil is in the embryo.

TABLE III
PERCENTAGE COMPOSITION OF FLAXSEED^{a,b}

Constituent	Whole seed (%)	Embryo		Hull	
		Full-fat (%)	Fat-free ^b (%)	Full-fat (%)	Fat-free ^c (%)
Moisture (5 hours in vacuum oven at 105°)	7.13	4.31	—	7.89	—
Nitrogen	4.01	4.64	10.92	3.18	3.52
Oil	38.7	53.20	—	1.84	—
Ash (4 hours at 550°)	—	3.38	7.95	2.99	3.31
Weight fraction	—	58.60	40.04	41.40	59.96
Per cent of total oil		96.7		3.3	

^a Taken from A. K. Smith, V. L. Johnsen, and A. C. Beckel, *Ind. Eng. Chem.* **38**, 353 (1946).

^b On hand-decorticated seed.

^c The oil- and moisture-free data are calculated from the full-fat analysis.

Linseed protein was first investigated by Osborne (84) in 1892. Vassel and Nesbit (85) studied the proteins of the flaxseed and proposed the name “linin” from the botanical name *Linum usitatissimum*, for the major protein component, and “conlinin” for another identifiable globulin. Smith *et al.* (83) investigated the yield and properties of the protein isolated from flax meal by alkali extraction and acid precipitation. They reported that the mucilage and the dark-colored pigments occurring in the hulls are water-soluble, and objectionable factors in protein isolation; the mucilage interfered with settling of the precipitated protein, and the pigments reacted with the protein to give it an undesirable color. They worked on a mechanical method of separating the hulls from the meats by passing the solvent-extracted meal (at 8% moisture or above) between smooth rolls followed by centrifugal separation in a Raymond Whizzer and screening to yield a meat fraction containing about 48% protein. On extracting this protein-rich

fraction with sodium hydroxide, clarifying the protein dispersion in a centrifuge, and precipitating with acid, the protein yield, based on the weight of the decorticated meal fraction, was 38%.

More recently Schlamb *et al.* (86) described an improved method for separating linseed hulls and cotyledons by flotation with petroleum ether as the flotation medium. Their separations were made for the purpose of investigating the nutritional value of the fractions. Properties important to the industrial utilization of the protein isolate were not reported.

The nitrogen content of the flaxseed proteins varies with the method of isolation. Alkali-extracted and acid-precipitated protein give the highest protein yield but the lowest nitrogen content of about 14%. The proteins isolated by salt solution followed by precipitation had nitrogen values ranging up to 18.05%. The results indicate that the nitrogen factor for calculating protein should depend on the method of isolation and would range from 7.14 for alkali-extracted and acid-precipitated protein to 5.5 for salt-extracted protein. The isolated flaxseed protein is dark in color and not readily dispersible in mild alkaline solutions.

From the research describing the isolation and properties of flaxseed protein, it appears doubtful if a flax isolate will be produced competitively with other vegetable protein isolates now on the market.

5. Sunflower Seed

a. Source Materials

Sunflower seeds have a sweet taste; the oil is of good edible quality, light in color, and reported to be quite stable. The light color of the defatted meal suggests that the isolated protein might be nearly white.

The composition of the whole sunflower seed and of the hulls, kernels, and solvent-extracted meal is shown in Table IV. Milner *et al.* (87) examined the composition of twenty-eight samples representing four varieties of seed. Their data show a range of crude protein content ($N \times 6.25$) for whole seed of 18.04 to 21.40% and of oil from 27.47 to 30.78%. The hulls on the four varieties constitute from 39 to 46% of the whole seed, and calculations show that the dehulled and defatted meal will have a protein content ($N \times 6.25$) of 60 to 74%. (See also Chapter 19.)

b. Isolation and Properties

The earliest publications on sunflower seed proteins were by Ritthausen (88) in 1880, Vines (89) in 1893, and Osborne and Campbell (90) in 1897. Osborne's protein was dark because of a substance he called "helianthotannic acid," which, in more recent studies, Gorter

TABLE IV
COMPOSITION OF WHOLE SUNFLOWER SEED, DEHULLED SUNFLOWER SEED,
HULLS, AND DEHULLED SOLVENT-EXTRACTED MEAL^a

	Moisture (%)	Ash (%)	Nitrogen (%)	Crude protein ^b (%)	Oil (%)	Sugar (%)
Whole sunflower seed	—	3.45	3.42	21.4	30.78	3.79
Dehulled sunflower seed	—	4.07	5.12	32.0	48.80	6.14
Hulls	—	2.33	0.59	3.7	1.05	—
<i>Dehulled solvent-extracted seed</i>						
Hexane (b.p. 30°–60°)	9.46	8.42	9.68	60.5	—	—
Ethanol, cold	7.47	5.32	7.98	49.8	—	—
Ethanol, hot	10.20	7.35	10.92	68.3	—	—
Hexane, followed by absolute methanol	9.60	9.09	11.88	74.2	—	—
Hexane, followed by 70% ethanol	11.62	9.19	11.62	72.7	—	—

^a Taken from A. K. Smith and V. L. Johnsen, *Cereal Chem.* **25**, 399 (1948).

^b Nitrogen \times 6.25. A conversion factor of 5.4 was used in the original publication.

(91) identified as chlorogenic acid. Rudkin and Nelson (92) have given the structural formula for a chlorogenic acid derived from sweet potatoes and indicated its function in plants as part of the oxidase system.

The effect of variation in *pH* of the solvent on the dispersion and precipitation characteristics of the protein of sunflower seed meal, previously defatted with petroleum ether or with ethanol, has been reported by Smith and Johnsen (93). For the meal from seed extracted with petroleum ether, they found that 25% of the nitrogen, less than half of which exists as protein, was dispersed in the *pH* range of 3.0 to 7.0, but 100% was dispersed at *pH* 10.0 and above. The protein in alcohol-extracted meal was partly denatured and more difficult to disperse than that in hexane-extracted meal. The maximum yield of protein, based on solvent-extracted and dehulled meal, was 31.8%; if based on defatted and undehulled meal, it would be much lower. The proteins prepared by Smith and Johnsen from salt solutions had nitrogen contents of 18.69%, whereas the proteins prepared by alkali extraction and acid precipitation had nitrogen contents not exceeding 17.63%.

The protein extracted with alkali had a dark green to brown color because of the presence of chlorogenic acid. Removal of the chlorogenic acid with several common organic solvents was attempted but without practical success; 70% ethanol was the most efficient solvent. The presence of chlorogenic acid in the meal can be detected by the addition of sodium hydroxide to form a

bright, chrome yellow color. The yellow color changes to green when the meal or protein is exposed to air or oxidizing agents. At pH 9.0 the green color appears in 8 to 10 minutes; at pH 11.5 the color changes directly to brown. Appearance of the green and brown colors can be prevented by reducing agents such as dithionite salts, but the color will gradually reappear after the reducing agents are washed out and the protein exposed to the air. Results of the pH-dispersion studies indicate a reaction between the protein and chlorogenic acid in the pH range of 3.0 to 7.0 to form an insoluble product. At pH 10 and above, the complex appears to be dissociated; removal of the chlorogenic acid would be possible if a water-immiscible solvent for chlorogenic acid could be found for a liquid-liquid type of extraction at this pH value.

Joubert (94) described a method for removing chlorogenic acid by repeated extraction of the defatted meal at room temperature with 50% ethanol and then with cold water, with use of a Waring blender for breaking up the meal. The meal was then washed with acetone at room temperature and air-dried. This treatment was said to remove completely the chlorogenic acid to yield a meal from which 80% of the protein was extractable with salt solutions. Apparently Joubert did not determine the dispersibility of the protein in dilute alkaline solution.

VIII. CEREAL GRAIN PROTEINS

The ratio of protein to starch in corn and wheat is roughly 1:7; thus, it is apparent that in developing protein products from these cereal grains the utilization of the starch has an important economic influence on that of the protein. The proteins of corn and wheat have some factors in common such as high prolamins content, but they also have certain marked differences. For example, the proteins of wheat combine with water to form an elastic mass (gluten) quite different from that obtained with corn gluten or any other readily available cereal protein (95); wheat protein is also higher in glutamic acid (96, 97). These two special properties are mainly responsible for commercial isolation of wheat gluten and its utilization in protein fortification of bread and in making monosodium glutamate.

1. Corn Protein

a. Corn Fractions

The protein content of corn varies from 6.5 to 20%, although the average for the Midwestern states of the United States is about 8.9% ($N \times 6.25$) (98, 99). The corn kernel is made up of approximately 5.8% hulls, 81.4% endosperm, 11.4% germ, and 1.4% tip cap. On a moisture-free basis, the endosperm will average 86.4% starch, 9.4% protein, and 0.8% oil; the respective values for the germ are 8.2%, 18.8%, and 34.5%. Further detailed data on composition of corn components have been published by Earle *et al.* (100); the literature on

the composition of the mature kernel has been summarized by Cannon *et al.* (101). (See also Chapters 3 and 28.)

About 90% or more of the United States corn production (3 billion bushels) is used in animal feeds. Somewhat less than 10% is processed by several mechanical methods into its component parts for a variety of food and industrial uses. Approximately 95 million bushels or 3% of annual production is processed by the dry-corn milling industry into breakfast foods, corn meal, flour, and hominy grits for human food and for making fermented malt liquors. About 30 million bushels, or 1%, is used by the distilling industry (102), and 135 million bushels, somewhat more than 4%, is processed by the wet-corn-milling industry into starch, corn germ, and corn gluten. The starch is used as such or converted into various adhesives and sizings, particularly for paper and textiles (103-106) and for making corn sugar and glucose syrups. The germ from both the wet- and dry milling is processed for oil and meal.

The oil of the corn germ is produced by solvent extraction or screw-pressing and is used for salad and cooking oil, and the protein residue is used entirely for feeds. The gluten normally contains about 60% protein. By means of special destarching processes with enzymes or by acid hydrolysis, the protein content of the gluten can be increased to 70%, thus making it suitable for certain industrial applications and for production of monosodium glutamate.

b. Zein Isolation

The major protein of corn gluten is zein, a prolamin soluble in 60% to 90% ethyl or isopropyl alcohol and other organic solvents (107). It is the only prolamin presently isolated from cereal grains for industrial purposes.

Zein was first isolated and named by Gorham (108) in 1821. Between 1891 and 1920, Osborne and associates (109) published more on corn proteins than was published on any other vegetable protein during that period. An annotated bibliography on zein by Rathman (110) has covered the literature from 1891 to 1952. Research by the wet-corn-milling industry (111, 112) on methods for isolating zein led to its commercial production in 1938. Evans *et al.* (113) have described a convenient laboratory method for its preparation.

The zein content of corn varies from 1 to 8% (114, 115), and it is present in an amount which approximates a linear relationship to the total protein in corn; thus, high-protein corn is especially valuable for zein production. For practical purposes, however, it is usually stated that the potential yield of zein is 1 pound per bushel of corn. On the basis of wet-corn-milling capacity, it is estimated that no more than 5 or 10% of the gluten produced is processed for zein.

c. Utilization

Many uses of zein are dependent on its solubility in volatile organic solvents. These and other outlets include spirit varnish formulations (mixed with rosin) for replacement of shellac, special decorative and greaseproof coatings,

printing inks, cork binder, special adhesive applications, and textile fibers. It has also been demonstrated that zein may be used in making phonograph records. Research at the Northern Utilization Research Branch culminated in 1948 in the commercialization of a zein fiber by a wet spinning method (116, 117). This process is dependent on earlier work which defined the limitations for the dispersion of zein in aqueous alkaline solutions (118, 119). Zein is completely dispersed in aqueous sodium and potassium hydroxide solutions in a pH range of 11.3 to 12.7, but the amount dispersed decreases to nearly zero immediately outside this range; it is insoluble in calcium and ammonium hydroxide solutions. Methods for stabilizing zein fiber (120, 121) and a comparison of its physical properties with those of wool and of synthetic fibers have been published (122). Zein fibers are marketed in the United States under the trade name Vicara (123–125) and resemble wool in physical properties. Traill (122) has reviewed the chemistry and history of regenerated protein fibers from soybean, peanut, zein, casein, gelatin, egg white, and chicken feather proteins. He compared the properties of fibers from these materials with those of wool, silk, cotton, and several of the synthetic fibers.

2. Wheat Proteins

a. Gluten Isolation

The crude protein content of wheat is highly variable; it ranges from 7 to 21%. In the United States hard spring wheats will usually have protein contents in the range of 11.5 to 15.0%; soft red winter wheat in the range of 9 to 12%; and white winter wheat, 8 to 11.5%. Protein content of wheat is an important factor in estimating the baking quality of flour, a factor leading to extensive research on wheat protein which was summarized to 1944 by Bailey (126). It has been reported that wheat protein contains eleven albumins, two globulins, a glutelin, and a prolamin (127, 128). Gluten, however, which is a mixture of globulins, glutelins, and prolamins, with approximately fifteen non-protein materials, is the wheat protein of greatest commercial interest.

Most of the methods (129) for preparing gluten involve mechanical procedures for washing the starch away from the gluten, which then forms a cohesive but elastic mass. Chemical methods for isolating gluten, although developed on a laboratory scale, have not been brought into commercial operation. (See also Chapter 3.)

In the Alsation method, the whole grain is soaked for 1 to 2 days in warm water, then crushed, and the starch washed away for recovery on settling tables or by centrifuges. Slotter and Langford (130) modified the process by steeping the grain in sulfurous acid. Sulfurous acid reduces the cohesiveness of the gluten so that the conventional wet-milling techniques of the corn-processing industry can be used.

The Martin process, which utilizes low grade wheat flour as a raw material, has probably been used the most in commercial operation. In this process,

wheat flour is made into a dough with about 40% of its weight of water. Time is allowed for the gluten to hydrate, and the dough is then washed with water to remove the starch. The present method of drying gluten, to maintain its undevitalized state, is in a vacuum dryer.

The batter process (131) also employs wheat flour instead of whole grain. The wheat flour is made into a slurry with about 4 parts of water at 40°; this slurry is then disintegrated with the addition of water by a high-speed propeller to form gluten curds which are recovered on a screen. This method gives a high yield of starch.

Dimler *et al.* (132), starting with wheat flour, have reported a chemical method for separating starch and gluten. The flour is treated with 6 parts of 0.03 *N* sodium hydroxide solution to disperse the gluten and suspend the starch. The starch is separated from the slurry by centrifuging to yield 70 to 80% of the total starch. The gluten is then precipitated by neutralizing the solution with acid and recovered by centrifuging or filtration.

Balls *et al.* (133) and Tucker and Balls (134) have proposed a process for separating wheat flour into three fractions. They treat a water suspension of wheat flour with sulfur dioxide and stir vigorously; the impure gluten forms a foam on the surface, the impure starch settles to the bottom, and the amylases concentrate in a clear solution in the middle layer.

b. Utilization

The most important uses for gluten are for manufacture of monosodium glutamate and fortification of weak flours to produce high-protein bread. The economy of this use in bread, which requires undevitalized gluten, is doubtful, since the nutritive value as measured by protein efficiency is not measurably increased by this procedure, and within reasonable limits the improved baking qualities can be attained at lower cost by paying a small premium for high-protein wheats.

The monosodium salt of the L-form of glutamic acid has the unique power of enhancing the flavor (135, 136) of many foods. Also, it is this salt which is largely responsible for the flavoring quality of soy sauce. Monosodium glutamate was developed to a commercial stage in Japan during the 1920's under the name Ajinomoto and was first produced in the United States in 1934. Five companies in the United States are now producing glutamate with an estimated annual capacity of 18 million pounds.

The raw materials for glutamate in the order of their importance are Steffen's waste (137), a by-product of beet sugar refining, wheat gluten, and corn gluten; soybean meal has been used extensively in the Orient. The so-called Steffen's waste was, at one time, a disposal problem for the beet sugar industry, but now it is the cheapest raw material source for glutamic acid and accounts for an estimated 50% of production. The amount of wheat gluten used for making glutamate normally exceeds the amount of corn gluten employed for this purpose. The

approximate glutamic acid content of various source materials are wheat gluten 36%, corn gluten 24.5%, zein, 36%, peanut protein 19.5%, cottonseed protein 17.6%, soybean protein 21%, casein 22%, castor bean protein 19%, yeast 18.5%, rice protein 24%, and Steffen's waste 0 to 15% as the glutamine.

The materials, production methods, properties, and uses of glutamate as a flavor ingredient for foods have been described by Hall (138) and were reviewed at a symposium (139) sponsored by the Quartermaster Food and Container Institute for the Armed Forces, and Associates, Food and Container Institute, Chicago, in 1955.

Reitz *et al.* (140) have described a "gluten sulfate" prepared by addition of cold concentrated sulfuric acid to gluten. It has the unusual property of absorbing 100 to 300 times its weight of water to form a firm, odorless, tasteless, non-toxic gel which is suggested for pharmaceutical preparations. Mohammad *et al.* (141) have reported a phosphorylated derivative of gluten which has good gel-forming properties and is reported as cheaper to produce than the gluten sulfate. Fong *et al.* (142) have proposed the use of gliadin, obtained by extracting gluten with alcohol, for preventing soil redeposition in textile cleaning operations. Sagi (143) has described a method for isolating gliadin from wheat gluten and has demonstrated its whipping and foaming properties. Horan (129) has reviewed other literature on the uses of gluten and gliadin.

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CHAPTER 11

POTENTIAL USES OF ISOLATED OILSEED PROTEIN IN FOODSTUFFS

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I. INTRODUCTION

The preceding chapter describes existing commercial methods for preparing and using isolated vegetable protein. This chapter is intended to supplement the description of the present applications of isolated oilseed protein by some discussion of the great potential uses in various foods for man and in milk substitutes for young pigs and calves. Although what will be said is applicable generally to isolated proteins of good nutritional quality and, in particular, to various oilseed proteins, it deals primarily with soy protein. Soy protein is of exceptional nutritional quality, and economical raw material suitable for the preparation of the isolated soy protein is already available on a large scale.

Since the large commercial application of isolated oilseed protein in foodstuffs is something for the future, we shall be content merely to sketch the types of possibilities and their scientific and technological basis. The start which has already been made in the United States is discussed in Chapter 15.

Although edible oilseed protein is not now made on a large scale, it is not without background in historical use and even in modern factory production. As will be made clear later, the ancient Chinese bean curd, which is a major source of good protein in some parts of the world, is essentially isolated soy protein. Furthermore, inedible soy, peanut, and corn proteins for industrial uses have been manufactured on a large scale for some time. (See Chapter 10.) The manufacture of edible oilseed protein is fundamentally the same as the manufacture of the industrial protein, although it differs in many important details such as the avoidance during extraction of high alkalinity which lowers the nutritive value of the protein. The cost of edible protein ought to be only a few cents a pound more than the cost of inedible protein, a difference mainly due to the need of making edible protein in a dairy-

type sanitary plant. Such edible soy protein as has been factory-made in recent years—for egg white substitutes, whipping mixes, and other uses—has been relatively expensive because of the small scale of manufacture and the failure or inability in the past to start with soy meal whose protein is completely extractable. Soy meal which permits complete extraction is now available without premium cost.

We shall discuss the historical uses of oilseed protein by man; the composition of oilseed meals and the beneficial results achieved by separating the protein from the other components of the meal; the application of isolated oilseed protein to cereal foods and to foods like animal protein foods; the application to milk substitutes for farm animals and to the fortification of diets for the sick; the production of fermentation foods from plant protein material; the relative costs of animal and plant proteins; and finally, the possibilities of using the few relatively cheap animal proteins in ways much the same as the ways in which isolated oilseed proteins can be used.

II. PREPARATION AND ADVANTAGES OF ISOLATED PROTEIN

Conditions for consumption of oilseed protein by man. Peanuts are eaten by man usually as a pleasure food, not as a major source of protein. In general, man does not consume any great amount of soybeans, cottonseed, or peanuts either as such or after the mere removal of the oil. The only oilseed protein consumed by man on a large scale in any form is soy protein. This has been done for thousands of years in the Orient as a basic part of the way of life for large populations, but only because methods were discovered for converting soybeans into acceptable products by radical processing beyond the mere removal of oil. There is, on a much smaller scale, some similar processing of peanuts, which is mentioned in Chapter 16. Hardly any cottonseed is processed as a protein food. The possibility of using isolated chick pea protein for human food is discussed in Chapter 26.

Soybean products eaten by man. There are two main forms of traditional soybean products eaten by man: soybean curd and soybean fermentation products, such as soybean sauce, soybean cheese, and the Indonesian product, Tempeh, described in Chapter 9. To understand the nature of these products and their implications for modern technology, one must first be clear about the composition of soybeans and about why, as a result of their composition, they are not eaten directly by man on a large scale, even after removal of the oil.

Oilseed components and isolation of the protein. The soybean (like other oilseeds) has four components or groups of components, all present in major amounts: (1) fat, (2) protein, (3) insoluble, indigest-

ible carbohydrate, and (4) water-soluble, non-protein substances. To isolate the protein from the fat-free meal, first the protein is dissolved in water, usually with the help of a little alkali, then the insoluble carbohydrate is removed by filtration or centrifugation, and finally the protein is precipitated, much as protein can be precipitated from skimmed milk, either by the addition of acid or, in the Chinese fashion, by heat coagulation in the presence of calcium or magnesium salts. The water-soluble non-protein fraction, the so-called whey, is left in solution and rejected after collection of the protein precipitate.

Advantages of protein isolation. The isolation of the protein, in addition to concentrating the protein, has a number of other advantages. The elimination of the insoluble, indigestible carbohydrate, which has great swelling ability, removes a substance with undesirable physical properties; the swollen, insoluble carbohydrate makes any food into which it is incorporated mushy, and it produces an undesirable, indigestible mass in the digestive system of man and young non-ruminant animals. In addition to removing insoluble carbohydrate, which constitutes almost a third of the meal, the isolation of the protein, as discovered by Osborne and Mendel (1) in their pioneer studies of the biological value of soy protein, removes some substances harmful to non-ruminants, substances which can also be destroyed by heating of the meal. Finally, the isolation of the protein, when carried out by the best techniques, eliminates practically all the bad-tasting materials associated with soybeans; these are rejected with the fraction of water-soluble, non-protein substances.

Soybean curd. The ancient Chinese preparation of soybean curd by hot suspension, filtration, and heat coagulation is essentially a crude separation of the protein from the undesirable, insoluble carbohydrate and the bad-tasting substances. The curd so obtained is rich in digestible protein of high quality, is bland in flavor, is in the physical form of a soft gel-like mass, and has the quality, possessed by every basic foodstuff, of being suitable for repeated, daily consumption. The ancient Chinese discovery of the technology of producing curd was a great historic step in the direct utilization of oilseed protein by man and has made possible a great protein nutritional experiment on many millions of subjects.

Modern possibilities of utilizing isolated soy protein. The traditional manufacture of soybean curd, which is still carried out on a vast scale in the Orient, points the way to a modern technology of using soy protein for man based on the separation of the soy protein from the other components of the bean. The wonder is that the oriental experience has been so long neglected.

Isolated soy protein. The factory isolation of protein can improve on the traditional procedure in a number of ways. First, the factory isolation can be carried out with considerably less waste of soy material. Second, the final product can be stabilized by suitable drying and can thus, unlike wet curd, be made a basic commodity of commerce. When large production is reached, the cost will be 25 to 40 cents a pound, as is shown in Chapter 15, or a few cents for man's daily requirement of good protein. Third, in modern factory procedures, the materials with undesirable flavors can be removed with greater completeness than they can be removed by the crude traditional procedures. Finally, the isolated protein can be made industrially in physical forms suitable for various end uses, some of which will now be described.

III. USES OF ISOLATED PROTEIN

1. Fortification of Bread and Cereals

Bread can be vastly improved as a protein food and made into a true staff of life, in some measure by a number of ways already in practice, and to a much greater extent by the addition of isolated protein. Since only about a quarter, or at most a third, of the retail cost of bread goes for ingredient costs, the addition of a few cents worth of good protein to a pound loaf of bread, without an increase in the costs of production and distribution, ought to have only a small effect on the total cost of bread sold to institutions or subsidized by government.

The quality of the wheat protein in bread can be improved by the addition of synthetic lysine. But synthetic lysine at present costs more than lysine in soy protein, and it cannot either increase the total protein content of bread or contribute to physical qualities. (Fortification of bread with lysine is also discussed in Chapter 13.)

Soy flour itself can be added to bread, and this is now done. If too much is added, the bread has a considerably changed texture and flavor. (See also Chapter 10.)

Finally, skim milk powder can be added to bread with great benefit. But this powder is only 30% protein, the protein costs considerably more than soy protein, and casein, in high enough concentration and in its usual unmodified form, can make a product gluey. The usual casein gels soften on being heated, but a non-gluey, heat-stable casein gel can be produced by the proper use of calcium and heat (2). It may prove feasible by such a technique to obtain fortification of bread with high amounts of casein or total milk proteins without creating undesirable physical characteristics.

Supplementation with isolated oilseed protein seems much more

promising than the existing forms of protein supplementation just outlined. We venture to predict that, wherever bread is eaten in considerable quantity and it is desired to improve the protein part of the diet at low cost, breads will be made containing 5 to 10% of the wet weight as isolated protein. This in addition to any lysine fortification that becomes practical.

There are at present no serious technical obstacles to making bread a complete food, not only a calorie food. In the United States, bread is made a carrier of some of the vitamins and minerals removed in the preparation of white flour. In Britain, bread is also made a carrier of amounts of calcium not normally found in wheat. Similarly, bread, without having its essential taste and texture and mode of manufacture changed or without losing its appeal as a familiar component of the daily diet, can be a carrier of truly large amounts of good, cheap protein. The protein fortification question is now one of nutritional and commercial policy, not of technology.

Cereals of the type of American breakfast cereals can be made to contain 50% or more of their dry weight as isolated protein. Since there is no standard product character of a cereal, like the standard character of bread, the possibilities are without limit.

2. Products Like Animal Protein Products

Protein-rich, cereal-like products can be made any time they are desired without the invention of essentially new procedures. It just happens that the countries with both the necessary raw materials and the necessary research and technical facilities have had no compelling motives for producing protein-rich cereals. In contrast with the relatively simple production of protein-rich, cereal-type foods, the more sophisticated production from isolated oilseed protein of products with the interesting textures and flavors of animal protein foods does, in many cases, require quite new techniques. The difficulties of the technical problem vary with the character of the animal protein product it is desired to have the plant protein product resemble.

a. Products Like Milk Products

The imitation of relatively structureless ice cream, for instance, is comparatively simple. For a while during World War II, the Ford Motor Company, as a result of Henry Ford's interest in soybean products, sold soybean "ice cream" in its cafeterias. All the usual milk ingredients of ice cream were replaced by soy ingredients. "Ice cream" made from soy protein and soy fat, or modified fat, presents no great

textural problems, and its flavors are the standard flavors of dairy ice cream.

Just as butter and cream can be imitated by suitably processed and flavored plant fats, so potentially all the types of whole milk products containing milk protein as well as milk fat can be imitated by products based on the combination of oilseed fat and oilseed protein. The Chinese use soy milk as an effective infant food. Its flavor and character could no doubt be changed to suit Western tastes. The Chinese also have various cheeses made from soy protein and soy fat.

The people of the United States spend about half of its sixty billion dollar food bill on animal protein foods (3), and about half the animal protein eaten is milk protein. It is, of course, ridiculous to suppose that there can be, or that there ought to be, any rapid change in this overall pattern of the vast consumption of milk, whatever the technical possibilities. In other parts of the world, where the milk industry is not so well established and, in some instances, cannot be established on a large scale, the pressures for new plant protein foods may be greater, once the technical possibilities are understood.

b. Meatlike Textures

The physical character of milk products is fairly easy to imitate, because the texture of milk products is not very complex. In contrast, meat, even ground meat, does have a complex structure which is quite foreign to the simple texture of precipitated oilseed protein. Much development work will have to be carried out before it is learned how to produce any desired type of complex texture from oilseed protein. Recent patents (2), however, make it clear that many interesting textures, some of them quite meat-like in character, can be produced by suitable manipulation of isolated oilseed protein.

Fundamental to the imitation of such structure is the recognition of the structural role of chewy protein gels which combine strength with high water content and moistness. The chewy elements of imitation meat, and possibly of real meat, are relatively tough gels, much tougher than soy curd or the familiar gelatin gels. The binding elements, when they are gels at all, as they often are, are weaker gels. A binding phase, whether it has gel character or not, both holds together the chewy particles and helps create an inhomogenous meat-like structure. General conditions for the production of protein gels based on protein aggregation have been known for some time (4). The specific conditions for the production of chewy and binding gels based on isolated protein, and for their use in meat-like products, are described in recent patents (2).

To make plant protein products of a fibrous character like that of unground meat, one must, as would be obvious to anyone "skilled in the art," have fibrous protein elements; and fibrous elements of all sorts are typically made by extrusion. The crude use of extruded protein material was described in a brief note on some German work (5). More effective is the application of conventional textile-type plant protein fibers to meat-like products (6-8a). A new kind of edible protein fiber can be made by extruding gel precursor protein material and then converting the precursor filaments into gel filaments by the application of heat (2).

One can now say that the manufacture of a variety of sophisticated products resembling ground or unground meat in texture will be possible, and that some basic techniques for doing this have already been discovered. But detailed development work has only begun.

c. Nutrition and Flavor

Once high-grade plant protein has been isolated free of substances with undesirable flavors or other undesirable qualities, and once the protein product has been given an interesting texture, there still remain the problems, if an acceptable meat-like food is to be made, of providing the textured protein with good nutritional and flavoring qualities at low cost. The nutritional problem is largely that of supplementing plant protein material with vitamins and minerals which the plant material may lack. This nutritional problem is faced generally in the formulation of foodstuffs based on plant proteins and is discussed in other chapters (Chapters 8 and 9). The flavor problem has not been solved adequately as yet, but the indications are that it can be solved to a practical degree. Classical meat-flavoring techniques can be applied to plant protein products resembling sausage and the like. And it is likely that true meat-like flavorings, like most major natural food flavorings, will be made in the factory, for new and powerful methods of separation and analysis and the new study of reactions such as the browning reactions are being applied successfully in many food research laboratories to the study of many of the basic food-flavor problems. There are two quite different types of flavoring substances being discovered: first, simple substances, such as the sugars, the acids of fruits, and the sulfides of onion, which are themselves flavoring agents; and second, combinations of substances which are themselves not flavoring substances but which interact to form complex flavoring materials, usually by browning reactions hastened by heat. The flavors produced by cooking or roasting tend to belong to the latter group.

All told, it is now clear that it will be technically possible to bypass

the animal and to prepare in a factory plant protein foods which have many of the desirable qualities of animal protein foods. Animal protein foods will not be displaced. But plant protein foods will take on many new forms and will achieve a new measure of acceptability and use.

d. No Necessity for Perfect Imitation of Existing Protein Products

It should be emphasized that the plant protein products of the future, although they must be interesting and pleasant and nutritionally sound, need not be perfect imitations of existing products, which are themselves immensely variable. Cereals and cheeses, for instance, are so variable in flavor and texture that it is necessary in the creation of similar products based on oilseed protein only to stay within very wide traditional limits. Meat is consumed in many forms which are far different from unground meat in texture and flavor—for instance, the variety of sausages which account for 40% of the German consumption of meat. Plant protein products based on chewy gels, fibrous or not, can easily come within the family of ground meat products and be more attractive than many. Even fibrous plant protein foods need not be exactly like any specific type of unground meat but can have flavors and textures of their own and their own acceptability. Ham, beef, and veal have very different flavors. Meat and fish differ in both flavor and texture. Cooking of meat and fish can alter flavor and texture radically. So there is room for fibrous plant protein foods with characters of their own, so long as they are attractive and not too foreign in character.

It should also be emphasized that the addition of oilseed protein to a product need not downgrade the texture of the product. There are many products, including some ground meat products, whose textures could be upgraded by the use of oilseed protein in suitable physical forms.

3. Special Uses for Young Mammals and the Sick

a. Isolated Protein for Young Mammals

A major recent advance in the rearing of hogs has come from the discovery that young pigs can be weaned two weeks after birth instead of after the usual six weeks and that they can be put immediately on a solid diet. With this new type of management sows can have three instead of two litters a year, and the young pigs grow more rapidly than when dependent on an inadequate supply of sow's milk.

The protein of the solid diet is provided by skim milk powder made for animal consumption. Oilseed meal, with its indigestible carbohydrate, is unsuitable. But there seems to be no reason why isolated oil-

seed protein would not do. We expect that isolated oilseed protein will have a use in the feeding of young pigs and young calves, just as soon as it becomes readily available in suitable form at a low price and its value becomes familiar. If the young animals do not object to soy taste, it may not be necessary to complete the isolation of the protein; mere removal of almost all the insoluble carbohydrate by simple screening may be adequate.

b. Isolated Protein for Infants

Perhaps the most serious cases of human malnutrition occur in those regions of the tropics where infants are weaned on essentially carbohydrate foods. (See Chapter 9.) It is now realized that it is not practical to get milk, even skim milk powder, to these infants, except in a small proportion of the cases. Some local form of cheap, good-quality protein, largely free of indigestible carbohydrate, is needed. Perhaps the necessary products can be made locally from oilseeds available or potentially available in the tropics. It is not necessary, contrary to what is often supposed, that the infant protein food have the exact composition and physical characteristics of milk. And, as pointed out in Chapter 9, once the child reaches a certain age, the conditions for protein feeding become much less critical.

The technical problems of feeding young children under adverse conditions are not essentially different from those of feeding young mammalian farm animals with the utmost economy.

c. Isolated Protein for the Sick

Protein malnutrition can often exist among certain groups of sick patients in populations which as a whole consume ample high-grade protein. Sick patients may not eat enough of the animal protein foods provided, and, in any case, they cannot get the abnormally large amounts of protein which they sometimes need by eating ordinary foods high in water content. Technically, it is a simple matter to add even large amounts of protein to a patient's diet by the suspension of dehydrated concentrated protein in milk, soup, or the like. Dried eggs and dry milk protein have been used with success (9). Dry, isolated oilseed protein would probably be also suitable and considerably cheaper. There is surely no reason for a shortage of good protein in a hospital diet which is based either on cost or on the difficulty of administering the protein to the usual patient. In the use of soy protein in foods for the sick there may be some purpose in fortifying the protein with synthetic methionine, in which soy protein is lower than animal protein.

IV. COST OF ISOLATED PROTEIN

Cost of protein for man. In most of the Western world "good" protein is almost entirely animal protein, and the cost of animal protein, in the forms actually used, is almost always relatively high (10). For instance, in the United States (1956) the 3.5% protein in milk at 23 cents a quart is \$2.48 a pound of protein; the 16.2% protein in meat at 46 cents a pound is \$2.58 a pound of protein; the 35.6% protein in non-fat, dry milk at 40 cents a pound is 97 cents a pound of protein; and so on for fish and eggs (11). It is not surprising that in large parts of the world, especially in Asia and Africa and parts of Central and South America, only little animal protein can be consumed. In Europe, before World War II, meat was eaten by many people only once a week, and, in general, the consumption of animal protein went very much with economic status. (There have been radical changes in recent years.) During World War II, the very lives of Europeans were saved in certain parts by the shipment of animal protein from the United States, but in other parts by an effective shift from an animal economy to a plant economy.

In contrast, basic isolated soy protein, at 25 to 40 cents a pound at the factory, is cheap, and mere fortification of accepted carbohydrate flour, which must be at least twice as cheap as isolated soy protein, on with as much as 10% of its wet weight as good soy protein, would be less than the usual cost of delivering the bread to the store.

Limitation of soy flour. A few remarks about the limitations of soy flour, which must be at least twice as cheap as isolated soy protein, on a protein basis. Although fortifying bread with soy flour is obviously cheaper than fortifying it with isolated protein, the amount of fortification possible with soy flour is one-fourth to one-third the amount of fortification possible with isolated protein. Many bread eaters of the world, furthermore, can afford the still low cost of isolated soy protein. For the very poor, in some parts of the world, who want to eat large amounts of soy proteins as a major source of good protein, the consumption of large quantities of soy flour in any form is impractical, as shown by the experience of the Chinese.

We see, then, that the basic agricultural cost of merely providing the essential nutrients for man is not high. Non-ruminant animals with nutritional requirements similar to those of man can be fed cheaply. During the 1930's, as is well known, much dog food was eaten by man in certain regions of the United States. Unattractive as this dog food may have been to man, it probably was often better from the nutritional point of view than the diets it replaced. Pellagra, at the time, was more common in man than in dogs fed on reputable brands of dog food.

Thus it is not good protein *per se* which is expensive or even good protein upgraded by isolation and put in simple acceptable foods such as bread and cereals. It is the process of using an animal to manufacture a concentrated form of protein free of undesirable substances, of pleasing texture and flavor, and well fortified with accessory food factors, which is expensive. This manufacture, which is expensive when carried out by animals, will also be expensive when carried out in the factory, although probably much less so.

In short, the experience with the feeding of non-ruminant animals and the experience of the Chinese with soy products shows that the basic cost of good protein nutrients is low, at least by the standards of even the poorest people of most technologically advanced states. But a high price must be paid at present for eating pleasure and for preferring foods which in the past were the only ones available providing good protein nutrition in acceptable forms.

V. FERMENTATION PRODUCTS

It has already been pointed out that soybean protein is eaten by man in the Orient not only in the form of curd, which is essentially isolated protein, but also in the form of various fermentation products. These products, like similar fermentation products made from fish, and unlike soybean curd, add flavor and accessory food factors to bland carbohydrate foods such as rice.

Hardly a beginning has been made at exploring the modern technological possibilities of manufacturing fermentation products based on oilseed meal or on fractions of the meal. One can be sure, at the least, that many types of cheese could be made, some resembling familiar milk cheeses in character. One Chinese soybean cheese, indeed, has a flavor strikingly reminiscent of the flavor of Roquefort.

VI. CHEAP ANIMAL PROTEINS

There are two relatively cheap forms of animal protein, neither of them conventionally used in amounts comparable to the amounts of other major animal proteins, fish protein in certain fish products, and the protein of skim milk powder. The possibilities of making interesting products from cheap animal proteins are mentioned because any advance in the technology of the use of any isolated protein would stimulate an advance in the related technologies of the use of other isolated proteins.

1. Fish Protein

The cheap form of fish protein is dried fish meal. This meal can be inexpensive if made in parts of the world where fish is extremely cheap

and in good round-the-year supply, or where it is made from fish material not usually used for consumption by men, such as the fish flesh left after filleting. Techniques for making cheap, bland, stable fish meal suitable for man are only now being perfected. And the potential amount of cheap fish protein which would possibly be made available is much smaller than the potential amount of isolated good oilseed protein.

2. Skim Milk Powder

The market price of skim milk powder is artificially low because of the artificially high price of butter fat. At that, the protein of skim milk powder is at least two and a half times as expensive as isolated soy protein. It should be possible to isolate the total protein of skim milk by the method the Orientals use to isolate soy protein, and this milk protein, like concentrated fish protein, could be used in ways similar to many of the ways in which isolated soy protein can be used. Isolated oilseed proteins have their own detailed properties, but their general properties do not depend on their being plant proteins. There could be an over-all technology of isolated proteins, plant or animal.

A vast amount of milk protein which it is not economical to collect is now fed to animals on the farm who could equally well use plant protein. Not many years ago the milk protein fed to animals in the United States was about equal to the combined beef and pork proteins consumed by man (12). Perhaps methods of isolating milk protein could be devised which could be applied locally on a small scale.

Large amounts of "surplus" skim milk powder have been distributed free all over the world. This program, with which there can be no economic competition, has stimulated the use of good protein for fortifying diets and thus has been of great importance in indicating ways in which isolated plant protein can be employed. It is unfortunate for many applications that the milk protein, which is only 30% of skim milk solids, is not separated from the lactose before being dried.

VII. SUMMARY

Isolated oilseed protein, which is free of bad flavor and undesirable insoluble carbohydrate and is of good nutritional quality and low price, can be put into a variety of foods for man, some resembling common cereal foods, others resembling animal protein foods. Wherever the people cannot afford animal protein, the simplest and cheapest uses of isolated oilseed protein will come into practice, as has happened in China and other parts of the Orient. Wherever the people are accustomed to and can afford readily available animal protein products, there will be

developed, in addition, more sophisticated plant protein products which will have to match the animal protein products in textural and taste appeal, and yet have advantages in price, in stability, or in some other important way to be competitive. Finally, the oilseed fraction free of fat and of insoluble, indigestible carbohydrate is potentially useful in milk substitutes for young pigs and calves, and in the diets of many sick people.

Given Nature's supply of a cheap plant protein of high protein nutritional value, the technologist and the factory can do many of the manufacturing jobs that have been done, at high cost, only by animals. The technical possibilities of bypassing the animal have been realized so recently, however, that no one can tell to what extent and in what myriad forms they will be put into practice, or how long it will take for a vast new industry to be established. My own opinion is that, just as the conversion of oilseeds to oilseed meals through processing created a revolution in animal feeding, so the further processing of oilseed meals into isolated protein and into complex foods containing isolated protein will create a second revolution. The millions of tons of oilseeds throughout the world, the basic complex raw materials which neither man nor beast can synthesize cheaply, are readily available as a starting point for the new technology.

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CHAPTER 12

CHEMICAL SOURCES OF NITROGEN AS SUPPLEMENTS TO PROTEIN FEEDS

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I. INTRODUCTION

This chapter summarizes the present information regarding the various nitrogen-containing compounds, other than true proteins and amino acids, which have been studied as potential nitrogen sources in animal feeds. An attempt has been made to bring together in a condensed summary the pertinent research findings and to relate this information to practical problems in feeding ruminants. Only selected references have been cited, preference being given to more recent research rather than to studies having primarily historical significance. For a fuller understanding of the early development of knowledge in this area the reader may consult the reviews by Krebs (1) and Reid (2).

The practical significance of chemical sources of nitrogen is limited to ruminant animals such as cattle and sheep because simple nitrogen-containing compounds are not useful to non-ruminant animals such as pigs, poultry, and horses as a replacement for dietary true protein or amino acids. The difference is explainable on the basis of the anatomy of the digestive tracts of the animals concerned. The presence in ruminants of the rumen and reticulum wherein conditions are ideal for fermentation of the food before it passes into the true stomach (abomasum) for enzyme digestion and the small intestine for absorption of all soluble materials dictates that all dietary nutrients are available for microbial growth. Only after fermentation has occurred is the food eaten by the animal actually available for use by its tissues. In animals with simple stomachs the food passes directly into the stomach and small intestine where it is digested and the soluble portion absorbed. The insoluble fraction is moved into the cecum and large intestine where varying amounts of bacterial activity occur, but only after all soluble nitrogenous compounds, such as urea or ammonium salts,

have been absorbed in the small intestine. The urea and ammonia so absorbed are excreted by the animal with the urinary waste products. Fermentation in the cecum and large intestine is important in the nutrition of horses, rabbits, and similar herbivorous animals because it permits partial utilization of cellulose and related carbohydrates and of less-soluble proteins, but there seems to be no important synthesis of amino acids and proteins.

II. MECHANISM OF NITROGEN UTILIZATION IN RUMINANTS

It is generally accepted that microorganisms in the rumen convert simple nitrogenous compounds into proteins for their growth and multiplication, and that they are later digested and utilized by the host animal (3, 4). Most if not all of synthetic activity is usually credited to bacteria. Progress is only now being made in elucidating the metabolic importance and interrelationships of the various rumen microorganisms. Thus, much remains to be learned regarding the kinds and specific requirements of the bacteria concerned in rumen function.

There is evidence that true proteins in the feed are partially broken down by bacteria in the rumen and the ammonia liberated is used in the formation of bacterial protein in the same manner as that arising from urea or ammonium salts. In cattle fed natural feeds the saliva which flows into the rumen in large volumes contains a trace of urea. Thus, it seems certain that bacteria adapted to utilization of urea would always be present in ruminant animals.

1. Compounds Useful as Nitrogen Sources

Several nitrogen-containing compounds have been tested as possible replacements for protein feeds. Urea has been more extensively studied and used as a protein substitute than any other nitrogen-containing compound. Ammonium bicarbonate, ammonium carbamate, ammonium acetate, ammonium lactate, ammoniated feeds (beet pulp, molasses, and certain industrial by-products), dicyanodiamide, glycine, and other compounds have been studied. A list of these various chemicals and some of their properties are presented in Table I.

2. Urea Nitrogen Is Not Utilized by Non-Ruminants

The non-essential amino acids can be synthesized within the tissues of all animal species. Amino nitrogen of the amino groups from other amino acids, or ammonia from urea or ammonium salts has been shown to serve for this purpose. But this process takes place only when the diet contains an ample supply of each of the essential amino acids. Since the amounts of the essential amino acids in natural feeds deter-

TABLE I
NITROGEN-CONTAINING COMPOUNDS TESTED AS SUPPLEMENTS FOR RUMINANTS

Compound	Formula	Nitrogen content (%)	Protein equivalent ^a (%)
Urea	$(\text{NH}_2)_2\text{CO}$	47	294
Ammonium bicarbonate	NH_4HCO_3	18	112
Ammonium lactate	$\text{CH}_3\text{CHOHCO}_2\text{NH}_4$	13	81
Ammonium carbamate	$\text{NH}_2\text{CO}_2\text{NH}_4$	36	225
Ammonium acetate	$\text{CH}_3\text{CO}_2\text{NH}_4$	18	112
Dicyanodiamide	$\text{NH}_2\text{C}(:\text{NH})\text{NHCN}$	67	419
Glycine	$\text{NH}_2\text{CH}_2\text{CO}_2\text{H}$	19	119
Glutamine	$\text{NH}_2\text{CO}(\text{CH}_2)_2\text{CHNH}_2\text{CO}_2\text{H}$	19	119
Biuret	$\text{NH}_2\text{CONHCONH}_2 \cdot \text{H}_2\text{O}$	35	219
Oilseed meal ^b		6.9	43

^a Calculated on the basis that protein contains 16% nitrogen.

^b Cottonseed or soybean oil meal, for example. This is included in the table to serve as a basis of comparison with the nitrogen-containing compounds. The nitrogen content of meals varies with the commodity and method of manufacture, and so will, of course, the protein equivalent.

mine their value as a source of protein, it follows that non-protein nitrogen compounds would not be expected to exhibit any protein-sparing action for non-ruminants fed natural proteins. Such has proved to be the case. Experiments with chickens (5), rabbits (6), and pigs (7) have all failed to demonstrate any protein-sparing effect from the addition of urea to a low-protein diet. These reports are in agreement with earlier studies in Germany and England.

III. FACTORS INFLUENCING THE UTILIZATION OF NON-PROTEIN NITROGEN

Many experiments in Germany before 1935 had produced evidence both for and against the usefulness of urea and other non-protein nitrogen-containing compounds as animal feeds. Studies in England (8) and Wisconsin (9) clearly demonstrated the value of urea in stimulating the growth of dairy cattle fed rations low in protein. Many experiments in the United States and Europe since 1940 have shown that urea and ammonium salts will spare protein for ruminant animals under many conditions, but with certain types of rations urea seems to have little or no value. Such factors as species and age of animals, type of carbohydrate, the level of true protein in the diet, and the adequacy of the diet with respect to sulfur and various other mineral elements seem to have important influences on urea utilization.

1. Influence of Age of Ruminants on Urea Utilization

It has been pointed out that non-protein nitrogen sources can replace true dietary protein only for ruminants. At birth the digestive tracts of cattle and sheep function like those of simple-stomached animals. Only after dry feed is eaten and digested with the resulting establishment of rumen function can amino acids be synthesized from urea. The age at which this takes place may be variable, depending on the diet, but it usually begins at 3 to 6 weeks. Brown *et al.* (10) compared as nitrogen sources for young dairy calves a low-protein calf starter ration with mixtures containing either urea or linseed oil meal fed at levels which supplied approximately 54% of the total nitrogen in the concentrate mixture. Timothy hay was fed free choice. The three groups of 12 calves each grew at comparable rates during the first period when a whole fluid milk was fed. After the milk was withdrawn at 49 days of age the calves grew very slowly on the low-protein mixture, which contained only 6.7% of crude protein. The average daily gains in weight of the calves from 2 to 86 days of age were 0.65 lb. on the low-protein, 1.10 lb. on the urea, and 1.16 lb. on the linseed oil meal-supplemented feed. The small differences in the averages for weight gain and height at withers between the groups of calves fed urea and linseed oil meal were not statistically significant, but the urea-fed calves digested significantly less of the crude fiber in the ration than did the calves fed linseed oil meal. The digestibility of the crude protein and crude fiber of the low-protein ration was much lower than the others. In this same experiment (10) half of the calves in each group were given cud inoculations each week for the first 6 weeks. The inoculations had no significant influence on urea utilization or on the growth rate or feed utilization for any of the diets. The authors concluded that a portion of the nitrogen requirements of calves 6 to 12 weeks of age can be supplied by urea. These results extend the earlier findings of Loosli and McCay (11) that calves utilized urea at 2 months of age.

2. Influence of Carbohydrates

The kinds of carbohydrates in the feed seem to have a marked effect on the efficiency of nitrogen utilization from non-protein sources. Cattle or sheep fed roughage alone have usually shown little or no response to urea feeding, nor have mixtures of urea and molasses or other forms of sugar given satisfactory results when fed together with roughages. The best results were obtained with rations containing the cereal grains. Bacteria in the rumen utilize carbohydrates as an energy source. But energy becomes available too rapidly from sugar (molasses)

and too slowly from cellulose (roughage) for efficient bacterial utilization of urea. Starch (cereal grains), being intermediate, promotes the most efficient use of non-protein nitrogen sources. This general concept is illustrated by citing results of two typical experiments. Tillman *et al.* (12) observed that when urea was dissolved in cane molasses to give a mixture containing 10% urea and 90% molasses and the mixture fed to replace cottonseed meal at a level to supply 42% of the dietary nitrogen, the rate of gain of fattening beef steers was slightly depressed and more feed was required per pound of gain. Addition of a small amount of corn improved performance. Similar results were obtained with yearling dairy heifers in studies at Cornell University (13). A supplement of soybean oil meal, with either corn or cane molasses, produced faster gains in weight when fed with low-quality timothy hay than did a supplement of urea and molasses. The addition of a little corn to the urea-molasses ration improved the average gain. A ration of good-quality legume hay, urea, and molasses gave fully satisfactory results. These results confirm the earlier reports from Wisconsin (14, 15).

Recently the use of *in vitro* techniques (16) has added to our knowledge regarding the role of specific carbohydrates on urea utilization.

3. Effect of Level of Protein

The view is generally accepted that the extent of utilization of non-protein nitrogen is inversely related to the level of dietary protein. At protein intakes well below the amounts required by ruminants there is ample evidence that simple nitrogen compounds are readily converted to protein, as already cited. The question as to whether a small amount of true protein is needed for the utilization of non-protein nitrogen compounds has not been answered. Evidence from *in vitro* incubation techniques by Burroughs *et al.* (17) suggests that ammonia nitrogen is fully adequate. Pearson and Smith (18), however, had found earlier that certain amino acids promoted, and others depressed, protein synthesis from urea. The results of these *in vitro* tests have not been verified adequately *in vivo* with animals.

Wegner *et al.* reported that the amount of urea converted to protein decreased as the level of casein was increased in the medium used for *in vitro* fermentation with rumen microorganisms. By analyzing a series of samples of rumen contents obtained from a fistulated dairy animal these same workers (19) observed that when the protein content of the concentrate fed was 18% or more the extent of conversion of added urea nitrogen began to decrease. On a grain mixture having 11.3% of protein, as much as 4.5% of added urea was utilized. Since

urea and ammonium salts can only supply nitrogen, they should never be used unless the ration is otherwise deficient in protein nitrogen.

4. Other Factors

a. Mineral and Organic Materials

There are other factors which influence the utilization of non-protein nitrogen in the rumen. These are thought to involve nutrients needed for the growth of specific microorganisms. Mineral elements which have been shown to be needed for utilization of non-protein nitrogen or breakdown of cellulose include sulfur, phosphorus, cobalt, copper, calcium, iron, and perhaps others. The ash of alfalfa has been shown to stimulate the activity of rumen microorganisms and cellulose digestion under certain conditions (20). This result has been confirmed with animals fed poor-quality roughage. Certain organic factors also are necessary for growth of rumen bacteria. Bryant and Doetsch (21) reported that *Bacteroides succinogenes* isolated from the rumen required two organic components for growth. Any of the branched-chain saturated acids, isobutyric, isovaleric, or DL- α -methyl-*n*-butyric acid can be used as one component. Any of a number of C-5 to C-8 straight-chain saturated fatty acids can serve as the second component. Bentley *et al.* (22) had shown that certain short-chain fatty acids stimulated cellulose digestion. The question as to what importance these findings may have in the practical utilization of non-protein nitrogen and low-quality roughages has not been answered; however, Hungate and Dyer (23) found that valeric or isovaleric acid did not influence either the utilization of a high-straw ration by steers or the microbial activity.

b. Sulfur and the Utilization of Urea Nitrogen

The proteins of the animal body contain an average of approximately one part of sulfur to each fifteen parts of nitrogen. Sulfur occurs in natural feed proteins in the form of the amino acids cystine and methionine. In non-ruminant animals cystine can be synthesized from methionine which furnishes both sulfur and nitrogen in useful forms, but methionine cannot be synthesized and thus must be present in the food eaten. In ruminants both of the sulfur-containing amino acids can be synthesized by microorganisms in the rumen. Loosli *et al.* (24) demonstrated the synthesis of cystine and methionine in the rumen of sheep receiving only urea nitrogen and inorganic sulfate as supplements to a nitrogen-free diet. In the absence of sulfur, urea was not utilized and the animals were in negative sulfur and nitrogen balance

(25). Block and Stekol (26) followed radioactive sulfur from the feed into the amino acids of the milk of a cow showing directly the pathway of inorganic sulfur through the rumen microorganisms into the methionine and cystine of milk proteins. Albert *et al.* (27) obtained fastest growth on a sulfur-low purified diet containing 4% of urea as the nitrogen source when they added one of the following: 0.64% of methionine, 1.27% of sodium sulfate, or 0.47% of elemental sulfur. Although elemental sulfur was utilized, 70% less total sulfur was needed when methionine was added, and 50% less when sodium sulfate was added.

(1) *Supplements of inorganic sulfur.* It has been suggested that a source of sulfur should be added to the feed when urea or other non-protein nitrogen is fed. Attempts to demonstrate an increased response to urea from feeding a sulfur supplement were unsuccessful in experiments with lambs in Germany (28) and at Cornell University with lambs (29) and dairy cows (30). In Oregon (31), however, adding 1% of sodium sulfate to raise the sulfur content of the ration to 0.13% increased the rate of gain of heifers approximately 10%. The difference is probably explainable on the basis of the sulfur content of the feeds. It is interesting to note that fertilization with sulfur increases both crop yields and the sulfur content of the plants. Sulfur occurs in plants as inorganic sulfates, as well as in the cystine and methionine of the protein molecule. Since the inorganic sulfates of plants can be used by the rumen bacteria for protein synthesis, it is the ratio of total sulfur to nitrogen in the entire ration which is important. The sulfur content of roughages may vary widely, and present data are inadequate to evaluate the possible response to sulfate supplements with urea-containing rations under all conditions. There is no evidence that a ration containing as much as one part of sulfur to each fifteen parts of nitrogen can be improved by additional sulfur.

(2) *Supplements of cystine and methionine.* The observation that wool is high in cystine content prompted attempts to stimulate the rate of wool growth by adding cystine to natural diets of sheep, with largely negative results (28). In contrast, a methionine supplement (32) increased the nitrogen retained by lambs on a diet in which approximately 40% of the nitrogen was supplied by urea. Hamilton *et al.* (33) found that cystine failed to improve the biological value of urea nitrogen for lambs. Similar studies have not been reported for cattle or goats. It is possible that wool growth results in a higher sulfur requirement for sheep than is true for cattle, but the comparative quantitative requirements are not known. In studies at Oklahoma (34) with fattening lambs fed a basal ration of prairie hay, shelled corn, beet pulp, and

minerals, supplements of urea or methionine added separately failed to increase the rate of gain. A combined supplement of urea and methionine improved gains slightly but not significantly, whereas soybean oil meal consistently improved rate of gain and feed efficiency. Adding methionine to soybean oil meal was without effect. From these studies it appears that methionine supplementation of rations for fattening lambs would not be profitable.

IV. LIMITATIONS ON THE USE OF NON-PROTEIN NITROGEN COMPOUNDS

General recommendations for feeding cattle and sheep suggest that urea or other non-protein nitrogen should constitute not more than approximately 25% of the total nitrogen in the feed. This general limitation has crept into common usage as the result of the poisoning of cattle fed excessive amounts of urea in the early days of its usage, and the fact that nitrogen utilization, growth rates of animals, and palatability of the feed sometimes decreased as the amount of urea was increased. Beyond the avoidance of toxicity, the level to be used should be governed by factors which influence the efficiency of nitrogen utilization and economic considerations.

1. Toxicity of Urea

An extensive study was made by Clark *et al.* (35) of acute urea toxicosis in sheep. The main symptoms described were dullness, hyperaesthesia, decrease or cessation of rumen motility, and bloating; twitching of the muscles, labored breathing, tetany, and death followed. The symptoms and death occurred within 30 minutes to 1 hour after as little as 10 g. of urea was administered directly into the rumen. On autopsy the kidneys and livers of all animals were found to be severely degenerated, with evidence of fatty infiltration of the liver. Cases of congestion and hemorrhage were seen in the endocardium, spleen, and small intestine. Acute circulatory collapse and generalized venous stasis were reported to have been the causes of death. Intravenous injection of large amounts of urea or ammonium hydroxide did not produce these effects. Administration of dilute acetic acid intravenously or into the rumen was an effective antidote.

Cattle drenched with 116 g. or more of urea showed ataxia, severe tetany, retarded respiratory rate, and excessive salivation within 20 minutes (36). Doses of 57 g. did not cause ataxia. No symptom of toxicity was noted among 8 steers fed 200 g. of urea in a mixed ration daily. In an early report from Wisconsin on a growing dairy animal fed a ration containing 4.3% of urea, kidney hypertrophy and damage were noted, but later studies have not confirmed this finding. Oklahoma

workers found that well-fed yearling steers refused to eat enough feed containing 8% of urea to produce toxic effects.

When urea is uniformly mixed with concentrates, levels that furnish 50% or more of the total nitrogen seem perfectly safe for cattle and sheep given adequate amounts of feed.

It has been suggested that urea is changed to ammonia in the rumen and that the rapid absorption of free ammonia causes the toxic symptoms. Hale and King (37) showed, however, that oral or intravenously administered ammonium carbamate ($\text{NH}_2\text{CO}\cdot\text{ONH}_4$) produced symptoms similar to those observed in acute urea toxicity. Intravenous injection of 5 g. of ammonium carbamate into a lamb caused ataxia, dyspnea, collapse, and tetany, but the animal recovered; 6 g. caused death of a 120-lb. lamb. In contrast, 5 g. of urea caused no ill effect. Injection of ammonium chloride caused relaxation of skeletal muscles rather than the spasms and tetany seen in urea and ammonium carbamate toxicity. These workers postulated that excess urea is converted in the rumen into ammonium carbamate causing toxicity in this manner rather than by ammonia absorption as formerly proposed. An alkaline pH is necessary for ammonium carbamate formation; it is destroyed in an acid medium.

It was reported (38) that urea as 3 to 12% of the medium had a bacteriastatic effect, but the possible effect on rumen bacteria has not been critically tested.

2. Rapid Availability of Urea

It has been shown that when urea is fed to ruminants ammonia is rapidly released and utilized as a nitrogen source for bacterial growth. Urea is highly soluble and rapidly broken down to ammonia in the rumen medium. After the feeding of urea all the nitrogen in the form of urea or ammonia has been shown to disappear from the rumen contents within 4 to 6 hours. Thus, it can be postulated that when urea constitutes a large portion of the dietary nitrogen fed in a concentrate mixture twice daily there may be a big surplus of available nitrogen for a few hours after feeding, followed by a progressive lack of nitrogen until the next feeding. The temporary surplus of available nitrogen may result in wastage, thus explaining the lower utilization of urea than expected, on the basis of its solubility. The periodic nitrogen shortage in the rumen medium may retard bacterial digestion of the fibrous components of feeds. On the basis of this reasoning it appears that an ideal source of non-protein nitrogen should be more slowly available than urea or ammonium salts so that its nitrogen would be released at a rate commensurate with the utilization of the carbohydrates as a

carbon source by the microbial population. The treatment of feeds with ammonia seems to hold promise of meeting this requirement. (See also discussion of effect of protein solubility on its utilization by ruminants in Chapter 16.)

V. AMMONIATED FEEDS

Methods have been developed of impregnating several agricultural products with ammonia to form chemical combinations of lower solubility than urea or ammonium salts (39, 40). Studies have been carried out using several ammoniated products to measure their practical usefulness.

1. Ammoniated Molasses

Several studies with beef cattle have shown that the addition of ammonia to cane molasses results in a palatable mixture furnishing available nitrogen and energy (41). In tests at Oklahoma, feeding an ammoniated molasses containing 32% of protein equivalent (nitrogen $\times 6.25$) produced extreme excitement in steers within 5 to 6 days, causing the animals to injure themselves. Molasses plus urea or ammoniated molasses, when sprayed on dried range grasses in the arid regions of the United States, has increased the consumption of the grass in some tests. Considering the cost involved, however, the practice has not given sufficiently good results to be accepted by livestock producers.

2. Ammoniated Feeds

Ammoniated sugar beet plup (42) appears to be a satisfactory feed. The use of ammoniated furfural residue (43) and ammoniated distillers' molasses solubles (44) to replace a portion of the protein supplement has resulted in little or no reduction in the rate of gain of cattle, but relatively little (10% or less) of the ration was replaced with the feeds being tested, thus making it difficult to evaluate accurately the feeds in question. Balance studies and *in vitro* tests at Cornell University (45) gave appreciably lower utilization of the nitrogen from an ammoniated furfural residue than from urea or soybean oil meal. Further study seems necessary to develop a product having satisfactory nitrogen availability.

VI. QUALITY OF PROTEIN SYNTHESIZED IN THE RUMEN

Cattle fed entirely on the corn plant, showed good growth and performance in spite of the known poor quality of corn protein for the rat and pig. Other studies have led to the general view that the quality of dietary protein is of relatively little importance for ruminants. Johnson

and Hamilton (46) observed that a considerable portion of the protein ultimately available to the ruminant is bacterial protein regardless of the nature of the nitrogen contained in the feed. Their studies led to the conclusion that rations containing 10 to 12% of crude protein (nitrogen \times 6.25) had a biological value of approximately 60% for sheep. Later studies from the same station reported biological values of 45 to 67% for rations containing urea or natural proteins. Lofgreen *et al.* (47), using sheep, found biological values of 71, 74, 76, and 80% for rations in which 40% of the protein equivalent was supplied by urea, urea plus methionine, linseed oil meal, and dried egg, respectively. These data show that not all dietary nitrogen is converted to a uniform bacterial protein, but that some food protein may be absorbed. Thus, the biological value of dietary protein appears to have importance for ruminants, although relatively little in comparison with non-ruminants. Hamilton *et al.* (33) concluded that the nitrogen of urea was as well utilized by growing lambs as were the same amounts of nitrogen from dried skim milk, or corn gluten feed, in rations containing 10 to 12% of protein equivalent and providing 16% of the nitrogen in the form of preformed protein.

VII. NON-PROTEIN NITROGEN IN LIVESTOCK RATIONS

In recent years urea has been used to some extent to increase the nitrogen content of low-protein feeds for sheep, beef cattle, and dairy cattle. The most successful results have been obtained with cattle. A few examples of recent research with cattle and sheep are cited to illustrate the practical potential of simple nitrogen compounds in saving protein feeds for use in the feeding of poultry and swine.

1. Sheep

In a ration for wintering ewe lambs Albert *et al.* (48) fed corn silage as the only roughage. Under these conditions a supplement of urea and corn sugar proved inferior in terms of average weight gains and wool growth to soybean oil meal which supplied an equal amount of nitrogen and energy. In feed-lot trials with fattening lambs, Willman *et al.* (49) reported that replacement of all the linseed oil meal by urea in a ration consisting of mixed hay, corn silage, and shelled corn resulted in decreased appetites of the lambs and slower rates of gain. The addition of cane molasses and sodium sulfate did not improve the performance of the lambs. The authors did not consider urea a satisfactory replacement for linseed oil meal. Pope *et al.* (50) observed that lambs gained 0.37 lb. per day on a ration of corn, dried beet pulp, prairie hay, and cottonseed meal, and containing 8.9% of crude protein.

Adding urea to give a crude protein equivalent of 11.6% did not result in an increased rate of gain, but the addition of extra cottonseed meal to equal the higher protein content resulted in an average daily gain of 0.40 lb. In a study at Michigan (51), the addition of urea or biuret to a ration containing 7.1% crude protein significantly increased the average daily gain of fattening lambs and decreased the feed required per pound of gain.

Experiments with pregnant and lactating ewes fed urea supplements have given results somewhat more consistently favorable than with fattening lambs (52).

2. Growing and Fattening Cattle

The value of urea as a replacement for cottonseed meal in the rations of cattle under various conditions has been tested in a long series of studies at Oklahoma (53). Under nearly all conditions urea has proved useful in replacing a portion, at least, of the protein supplement. In two of the trials, when urea was fed in a pelleted mixture the feed became somewhat unpalatable after long-time feeding.

A number of Agricultural Experiment Stations have tested the use of urea to increase the protein equivalent content of silage made from corn and sorghum. Most of the results have been in agreement with the report (54) which stated that corn silage to which 20 lb. of urea was added per ton of fresh cut forage was a palatable feed. The weight gains of beef cattle to which the urea-treated silage was fed were about equal to those of other cattle fed corn silage and soybean oil meal. Balance studies with sheep indicated that the nitrogen in the urea-treated corn silage was as well digested and retained as nitrogen from a soybean oil meal supplement. In Florida tests the use of 50 lb. of urea per ton of forage resulted in an unpalatable silage.

Beef cattle have also received urea under grazing conditions. The urea has generally proved to be useful when the cattle received some cereal grains, but the results have not been favorable for cattle on range grass alone. It is clear that urea and other non-protein nitrogen sources may be well utilized by beef cattle under favorable dietary conditions, and it is expected that the use of these supplements will increase in beef cattle feeding.

In a well-controlled study, Watson *et al.* (55) used long-time feeding periods with beef calves to show that urea was used for formation of protein tissue. Balance trials and slaughter studies were carried out in connection with the feeding trial. The cattle fed the basal diet (4.3% crude protein) gained very little weight; another group was fed casein and gained at a normal rate; and a third group fed equal

digestible nitrogen in the form of urea gained 70% as much. Slaughter studies showed that about 80% of the weight gained by the low-protein controls was fat, compared with 25% for the calves fed urea and 20% for those fed casein. It is clear that urea was utilized but was not so efficient a source of nitrogen as was casein.

3. Dairy Cattle

Prior to 1940 a number of experiments were carried out in Germany to compare the value of urea with protein feeds for milking cows. Some of these studies, as reviewed by Reid (2), showed that urea was efficiently utilized for milk production, whereas in others it was concluded that urea nitrogen was only 40 to 50% as valuable as the nitrogen in linseed oil cake and other protein feeds.

Owen *et al.* (56) in England observed that most of the cows fed urea maintained milk yield almost as well as those fed blood meal as a supplement. More recently Bartlett and Blaxter (57) concluded from a series of farm trials, that urea had a protein-sparing action when added to a low-protein ration, but that when a sufficient quantity was fed to justify its commercial use it depressed milk yield.

In the United States, Rupel *et al.* (58) found that the addition of urea to a low-protein ration for milking cows increased milk yield. The basal ration plus linseed oil meal resulted in slightly more milk than the urea-supplemented ration. Archibald (59) reported that cows fed a mixed supplement of cottonseed meal, soybean oil meal, and corn gluten feed with a ration of mixed-grass hay, corn silage, and beet pulp gave slightly more milk and maintained their body weights better than those fed urea as a supplement, but the differences were not statistically significant. In several other trials urea has proved to have important protein-sparing action, but often the milk production was slightly lower than on protein feeds. Recently a urea-supplemented ration for dairy cows gave milk yields similar to one containing soybean oil meal, whereas a ration containing dicyanodiamide and a low-protein basal ration gave slightly lower milk yields (60). Thus, from this study one might doubt that dicyanodiamide is equal in value to urea for milking cows, but further studies are necessary to evaluate its usefulness.

4. Extent of Use and Trends in the United States

There seems to be ample evidence that with concentrate mixtures high in cereal grains, as are used in the United States, urea is useful as a protein replacement, although it may not be fully equal to protein

feeds with all types of rations. In the United States urea is now widely accepted as a fully satisfactory feed ingredient for cattle.

The Feed Survey Committee of the American Feed Manufacturers Association estimated that in the twelve-month period beginning October 1, 1955, approximately 90,000 tons of urea was fed to ruminants. Urea contains about six times as much nitrogen as soybean oil meal or cottonseed meal, and it can be estimated, therefore, that urea is saving not less than 540,000 tons of high-protein feeds yearly at the present rate.

The question as to when it is economical to use urea depends largely on the market prices of urea, high-protein feeds, and corn or other low protein high-energy feeds. For example, a mixture of 1 lb. of urea plus 6 lb. of corn grain is approximately equal in nitrogen to 7 lb. of solvent-extracted soybean oil meal. When the cost of the soybean oil meal is lower than an equal weight of the urea-corn grain mixture, the soybean oil meal is the best buy. Similar comparisons can be worked out for other feed combinations. Of course, a short supply of high-protein feeds is also a factor at times. The energy value of feeds is as important a consideration as the nitrogen content. Urea contains no useful energy, and therefore when a protein feed is replaced by urea the energy value of the final feed mixture may be reduced slightly unless the low-protein feed added as a partial replacement is high enough in digestible energy to compensate. Considering the small quantities of urea normally used, the difference in energy value of the finished feed is usually not very important.

It is likely that the use of urea and possibly other simple nitrogenous compounds will continue to increase in the nutrition of ruminants.

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SUPPLEMENTATION OF PLANT PROTEINS WITH AMINO ACIDS

J. WADDELL

I. INTRODUCTION

It is a tacit assumption throughout this book that the nutritional qualities of a protein are determined by the amount and the kind of amino acids which it supplies to the animal organism. Allison (1) has stated: "The primary purpose of a dietary protein is to provide a pattern of amino acids appropriate for the synthesis of tissue proteins and for other metabolic functions."

In other chapters the principal sources of vegetable protein are discussed: their availability, their nutritional properties and how the latter may be affected by processing, and especially their value as sources of essential amino acids. In this chapter consideration will be given to the utility of certain synthetic amino acids for increasing the concentration of the most limiting amino acid(s) in the dietary mixture with the object of presenting a pattern more suitable to the animal's needs. It seems obvious that only under certain circumstances can synthetic chemical compounds be used to augment natural sources of amino acids; it is proposed to discuss the animal experimentation which has been carried out to define the circumstances under which such a procedure is beneficial and economical. The basic principle involved is that a small amount of a relatively expensive synthetic compound can produce a balance or pattern of amino acids which cannot be obtained otherwise or only at the expense of excessive use of other amino acids.

The data presented in Table I are of interest in determining which of the essential amino acids may be least abundant in the protein sources available for feeding man and animals (2-4). The point of main interest is that in the great majority of proteins, including the most important protein sources, the main deficiency is either lysine, methionine, or the combination of cystine and methionine. This may serve to explain why the discussion in the following pages centers mainly around the availability and utilization of methionine (or methionine-active analogs) and lysine.

TABLE I
AMINO ACIDS LIMITING THE NUTRITIVE VALUE OF FOOD PROTEINS^a

Food protein	Limiting amino acid	Deficit ^b	Chemical evidence ^c	Biological evidence ^d
<i>Animal foods</i>		(%)		
Beef muscle	Cystine and methionine	29	+	+
Horse muscle	Cystine and methionine	35	+	
Chicken muscle	Cystine and methionine	31	+	
Crustacean muscle	Cystine and methionine	28	+	
Heart	Isoleucine	35	+	
Kidney	Cystine and methionine	35	+	
Liver	Isoleucine	30	+	
Brain	Isoleucine	36	+	
Cow's milk	Cystine and methionine	32	+	+
Casein	Cystine and methionine	42	+	+
Lactalbumin	Methionine	34	+	
Human milk	Methionine	46	+	
Egg albumin	Threonine	22	+	
Gelatin	Tryptophan	100	+	
<i>Cereals</i>				
Whole wheat	Lysine	63	+	+
Wheat germ	Isoleucine	62	+	
White flour	Lysine	72	+	+
Gliadin	Lysine	86	+	+
Whole corn	Lysine	67	+	+
Corn germ	Methionine	61	+	
Corn gluten	Lysine	89	+	
Zein	Lysine	100	+	+
Oats, rolled	Lysine	54	+	+
Rice, white	Lysine	56	+	+
Rye	Lysine	42	—	+
Hegari	Lysine			+
<i>Legumes</i>				
Navy bean, <i>Phaseolus vulgaris</i>	Cystine and methionine			+
Lima bean, <i>Phaseolus lunatus</i>	Cystine and methionine			+
Adsuki bean, <i>Phaseolus angularis</i>	Cystine and methionine			+

^a The data in this table (somewhat revised) were taken from those of H. H. Mitchell, in "Proteins and Amino Acids in Nutrition" (M. Sahyun, ed.), p. 70. Reinhold, New York, 1948.

^b Percentage deficit in the indicated amino acid as compared with the proteins of whole egg.

^c Plus sign indicates existence of chemical evidence that amino acid named is the limiting one. Minus sign indicates that evidence concerning limiting amino acid named is based on biological evidence only.

^d Plus sign indicates existence of biological evidence that amino acid named is the limiting one.

TABLE I (Continued)

Food protein	Limiting amino acid	Deficit ^b	Chemical evidence ^c	Biological evidence ^d
Soybeans	Methionine	57	+	+
Peanut	Methionine	76	+	
Pea, field	Cystine and methionine	66	+	+
Pea, garden	Cystine and methionine	66	+	+
<i>Other vegetables</i>				
Potatoes	Cystine and methionine			+
Leafy vegetables	Methionine	44	+	
Alfalfa leaves	Cystine and methionine	40	—	+
<i>Miscellaneous</i>				
Cottonseed	Methionine	57	+	—
Cottonseed meal, auto-claved	Lysine			+
Flaxseed	Lysine	65	+	
Sesame seed	Lysine	61	+	+
Sunflower	Lysine	47	+	—
Yeast, brewers'	Cystine and methionine	55	+	+
Yeast, torula	Cystine and methionine			+

The identification of the most limiting amino acid(s) in a protein food is only a partial description of its nutritional value; it is necessary also to know those amino acids of which it is a good source. The data in Table II, prepared from those of Almquist (5), illustrate the value of the protein in some important feeds as a source of the amino acids most likely to be deficient in diets for growing chicks. Few of the protein sources could be used alone to provide the 20% protein required, but the method of calculation indicates those which carry their quota of an important amino acid and those which do not. Single protein sources are rarely consumed alone but are variable constituents of mixed diets. The function of the nutritionist is to combine them on the basis of composition, availability, and cost to obtain a total concentration of all the essential amino acids which will approximate the needs of the animal, using synthetic sources where it is difficult or impossible to attain adequacy of the less abundant amino acids.

II. AVAILABILITY AND PRODUCTION OF SYNTHETIC AMINO ACIDS

It has been reported (6) that processes exist for the commercial manufacture of all the essential (indispensable) amino acids. Indeed, the latter may be found listed in the weekly current market quotations (7), mainly as the DL-racemates, but the prices at which they are offered indicate that no large-scale production or use exists for any other

TABLE II
PERCENTAGE OF CERTAIN AMINO ACID REQUIREMENTS OF CHICKS SUPPLIED BY CERTAIN PROTEIN SOURCES IF USED TO PROVIDE 20% PROTEIN TO THE DIET^a

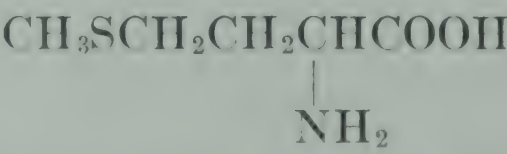
Protein source	Amino acid				
	Arginine	Lysine	Methionine	Cystine	Tryptophan
	%	%	%	%	%
Fish meal (60%) ^b	111	200	133	95	140
Fish solubles, condensed	71	102	76	34	89
Meat scrap (50%)	100	120	62	69	70
Skim milk	52	159	103	69	128
Soybean meal (45%)	104	138	61	84	129
Cottonseed meal	136	83	62	114	107
Peanut meal	144	66	44	91	109
Sesame meal	148	64	142	75	120
Sunflowerseed meal	137	94	151	91	131
Linseed meal	129	70	107	108	160
Yeast	74	153	58	69	122
Alfalfa (18%)	74	111	79	108	128
Corn gluten meal	52	37	97	92	50
Corn	74	74	69	95	89
Wheat (13%)	64	68	72	105	123
Wheat bran	94	83	47	68	131
Oats	83	74	48	105	117
Barley	69	56	44	95	108

^a Based on the content of the indicated amino acids in the feedstuffs shown and the requirements of the growing chick as estimated by H. J. Almquist, "Proteins and Amino Acids in Animal Nutrition," 3rd ed., U.S. Industrial Chemicals Co., New York, 1953.

^b Figures in parentheses denote content of crude protein in particular protein source.

than methionine and lysine. For limited use in nutritional and biochemical studies, isolation from protein hydrolyzates may offer the best method of production for some, as has been indicated recently for L-tryptophan (8). Synthetic DL-tryptophan was used as a supplement to protein hydrolyzates prepared by acid hydrolysis, but it is believed that this market is less now than it was some years ago. Therefore, we shall limit our consideration here to methionine (including the calcium salt of the hydroxy analog of methionine) and lysine, since, on the basis of actual or potential usage, they are the only ones of present interest.

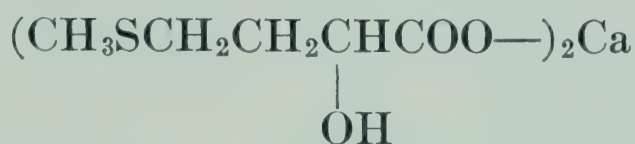
1. Methionine



In 1954, according to a report (9) based on Tariff Commission figures, there were produced in the United States around 1 million pounds

of DL-methionine. Some of this was for pharmaceutical preparations, but the large part was used as an addition to animal feeds. In the absence of accurate estimates for subsequent production it can only be stated that present annual production (1957) is much greater than the 1954 figure; in particular it is known that the use of DL-methionine, feed grade (purity 98% or better), as a supplement to animal feeds has increased markedly since then. There is believed to be a small amount of DL-methionine manufactured in Europe for pharmaceutical use.

2. Methionine Hydroxy Analog



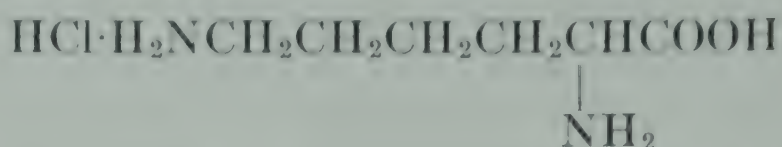
In 1952, Bird (10) published a brief account of an experiment comparing DL-methionine, DL- α -hydroxy- γ -methylmercaptobutyramide, and a salt of DL- α -hydroxy- γ -methylmercaptobutyric acid as to their ability to supplement a peanut meal-cerelose type of diet for growing chicks. The results indicated that the two "analogs" were approximately equivalent to methionine, when they were fed on an equimolar basis, in promoting growth and improving feed efficiency on the methionine-deficient diet. Gordon *et al.* (11) also reported that an addition of 0.05% methionine hydroxy analog (presumably the calcium salt, although it is not stated) to a corn-soybean oil meal type of diet for chicks, or to the same diet with a small supplement of fish meal or whey, resulted in a significant reduction in feed required per unit of gain. Since then, limited commercial use of this methionine analog has been reported, but the present or potential level of production has not been announced. The product is defined in the Official Publication of the Association of American Feed Control Officials (12) as methionine hydroxy analog calcium which contains a minimum of 95% DL-2-hydroxy-4-methylthiobutyric acid calcium salt.*

The presence of calcium (about 12%) in the hydroxy analog molecule together with the impurities would suggest that it should be only 80 to 85% as active physiologically on a weight basis as DL-methionine. Claims have been made, however, that it is equivalent, pound for pound, in nutritional experiments, but these have not been documented adequately in the scientific literature. An abstract of an oral presentation (13) suggests that the D-stereoisomer of the hydroxy compound is more efficiently inverted to the L configuration in the animal tissues than is the D-methionine. Sullivan and Bird (14) reported that, on a low-pro-

* Methylmercapto-, methylthio-, and methylthiol- are synonyms for the terminal $\text{CH}_3\text{S—}$ group on methionine and related compounds.

tein diet, the analog was not so efficient as methionine in promoting growth and improving feed efficiency.

3. Lysine



The situation as regards the commercial availability and methods of producing lysine has been summarized recently (15, 16). Three methods of production may be recognized—chemical synthesis, “biological” synthesis using selected microorganisms (17), and isolation from hydrolyzed lysine-rich proteins. The first two methods are considered to hold the greatest promise for large-scale production. Chemical synthesis, of course, leads to the formation of the racemic mixture, DL-lysine. Additionally, since lysine is quite basic in character and difficult to isolate as the free amino acid, it is prepared as a salt of some acid, generally hydrochloric acid. Both amino groups of the lysine molecule can be involved in salt formation leading to the dihydrochloride, but, more commonly, by properly adjusting the conditions of crystallization, the monohydrochloride is obtained and DL-lysine monohydrochloride is the usual first product of chemical synthesis.

It has been fully established that the unnatural D-lysine is without nutritional value. Interest, therefore, has been great in methods for separating the two stereoisomers; one such procedure for resolving the racemic mixture obtained by chemical synthesis has been developed. This leads to the production of L-lysine monohydrochloride in at least 95% purity (the impurity being D-lysine monohydrochloride), which is presently being manufactured on a limited commercial scale. Lysine prepared by biological synthesis, or by separation from protein hydrolyzates, is also offered as L-lysine monohydrochloride. All present (1957) production of lysine in the United States is going into special food and pharmaceutical outlets for human use at a price of around \$12 per pound. As will be shown later, certain animal diets are markedly improved by the addition of lysine, and much interest is expressed in the availability of lysine for feed use. No serious consideration can be given to such an outlet, however, until the price is very much lower. It is fully realized that if production could be expanded the price would decrease, but it is going to take time and much more information before the place of synthetic lysine in animal diets is clarified.

The hydrochloric acid moiety on both the DL- and L-lysine monohydrochlorides makes up almost exactly 20% of the molecule. Hence, only 40% of the former and 80% of the latter may be calculated as nu-

tritionally active lysine.* This is a further consideration in estimating the cost of lysine supplementation.

III. SUPPLEMENTATION OF DIETS BASED ON CORN AND SOYBEAN OIL MEAL

Corn is the principal cereal grain and soybean oil meal the principal protein concentrate used in the feeding of livestock in the United States. In formulating diets of different protein content it is general practice to substitute one ingredient at the expense of the other, since

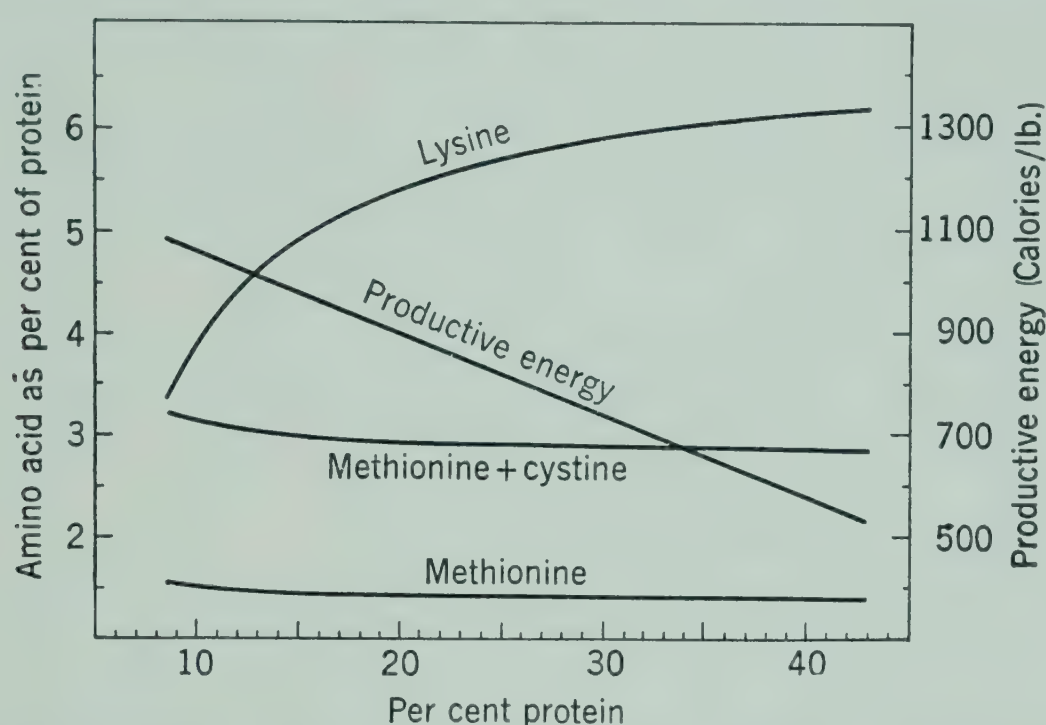


FIG. 1. Lysine, sulfur amino acids, and energy levels in mixtures of corn and soybean oil meal. These calculations are based on the assumption that the two ingredients make up 95% of the diet and on the following values (5, 23): corn—9% protein, 0.3% lysine, 0.14% methionine, 0.15% cystine, and 1145 Calories per pound; soybean oil meal—45% protein, 2.8% lysine, 0.62% methionine, 0.66% cystine, and 565 Calories per pound. The amino acids are plotted as per cent of total protein, the energy as Calories of productive energy per pound of diet.

supplementary items in the diet tend to remain relatively constant. Thus, as the protein level is increased the proportion supplied by soybean oil meal increases and that supplied by corn decreases; the converse is true as the protein level is decreased. For example, turkey starter diets of 28 to 30% protein content may have over 70% of the protein supplied as soybean protein, and broiler and chick starter diets of 20 to 22% protein content may have 60% or more as soybean protein. In swine rations or laying hen diets of 15 to 16% total protein, soybean protein may supply only about 40% of the total.

Figure 1 was constructed to illustrate the change in content of ly-

* Some authors, publishing in the nutritional literature, do not indicate clearly the basis on which a lysine supplement is calculated. The reader cannot determine whether the amount of supplement fed represents the hydrochloride or free lysine.

sine, methionine, and methionine plus cystine of the protein resulting from the mixing of corn and soybean oil meal in varying proportions. The effect of the soybean protein on the lysine content of the mixture illustrates its importance as a source of this amino acid. The change in the content of the sulfur amino acids with change in protein level is relatively small, although it is definitely shown that soybean protein is a poorer source of these amino acids than corn protein. The graph illustrates the fact that, as corn and soybean meal are varied in a diet, the protein will change in quality as well as in quantity; at low protein levels the mixture may provide a protein as limiting in lysine as in sulfur amino acids.

Also illustrated in Fig. 1 is the effect of changing proportions of the two ingredients on the productive energy content of the mixture. The phenomenon of energy content changing inversely with protein content in corn-soybean meal diets has not been well appreciated in the past. As will be discussed later, energy changes as well as changes in protein level and protein quality markedly affect diet performance and the problems of amino acid supplementation.

1. Supplementation of Chick Diets

Feed represents the largest single item in the cost of producing broilers. The rate of growth of the chicks together with the amount of feed required per unit of gain are the prime criteria by which feed quality is judged and cost estimated. Additional criteria are the quality of feathering, color, and vigor of the birds. There has been, therefore, great interest in all the nutritional findings that affect the growth of chicks, and the feed industry, which is highly competitive, quickly applies any new knowledge to the improvement of its mash formulations. The feeding of these broilers undoubtedly represents the greatest practical application of the science of nutrition.

There has been constant and striking improvement in the efficiency of broiler production, especially over the last decade. Much is made of the fact that by 1957 in the United States a 3-pound bird (average of both sexes) could be produced in 8 to 9 weeks on a total consumption of 6 to 7 pounds of feed as compared with 12 to 14 weeks of growing time and 12 or more pounds of total feed previously. Although the breeding of improved strains of chicks and better management must receive a share of the credit for the more efficient performance, the major portion must go to improved nutrition.

a. Broiler Diet

The broiler diet in the United States is based essentially on corn and soybean oil meal and such other additions as are needed to supply

vitamins, minerals, and unidentified growth factors. Vitamins, including vitamin B₁₂, are so easily available as commercial concentrates that with few exceptions no single natural ingredient is added primarily because of its content of known vitamins. Small amounts of animal protein, such as fish meal, fish solubles, and meat and bone meal are included, and, although their protein (amino acid) contribution is prized, it is safe to say that their content of unidentified growth factors is a more important reason for their use. Similarly, small amounts of dehydrated alfalfa meal and fermentation residues (distillers' solubles, whey, etc.) are used for the same purpose. Antibiotics and, in some cases, an organic arsenical are included for growth stimulation and "disease" control. Commercial broiler and growing mash regularly contain a coccidiostatic drug, but this is usually omitted in diets to be used under laboratory conditions where exposure to this infection is minimal.

Examples of modern diets, nutritionally adequate and capable of promoting rapid growth in young chickens, are shown in Tables III and IV. One (Table III) is the reference chick diet proposed by a committee of the Animal Nutrition Research Council (ANRC) as a positive control diet or reference standard for comparing experimental results either from one laboratory or from several laboratories (18). Considerations of availability and minimum variation in quality dictated in some degree the choice of ingredients and particularly the number of natural ingredients in this formula. The other (Table IV) is the Arkansas performance test diet (19), which is somewhat more typical of commercial mixtures. The formulas of the two diets will serve to illustrate the sources of nutritional supplementation for the corn-soybean meal type of diet, the different nutrients, and the levels at which they are included. Especially, they illustrate the way in which synthetic methionine is used as a supplement in such diets.

b. Dietary Energy and Nutritional Requirements

It has been only within recent times that poultry nutritionists have concerned themselves with the energy content of diets. In retrospect, it seems strange that such important considerations as the energy level in the diet and the energy intake of experimental animals should have received such scant attention, especially since the concept of food as a source of energy was the foundation of the science of nutrition. Clinical nutritionists have, of course, been more traditional in their approach because they usually describe diets in terms of calories from different classes of nutrients, and students of ruminant nutrition routinely use "nutritive ratio" in calculating dietary protein in relation to the energy-yielding nutrients. Even as long ago as 1923, Mendel (quoted in reference 4) stated that the energy content of a diet would influence the amount that an animal would consume under ad libitum feeding and hence influence the intake of specific nutrients. Also, the "sparing" ef-

TABLE III
REFERENCE CHICK DIET^a

Ingredient	Lb./100 lb.	Lb./ton
Yellow corn, ground (grade 2)	60.70	1214
Hulled soybean oil meal, 50% protein, minimum	27.98	559.6
Dehydrated alfalfa meal, 17% protein	2.00	40
Condensed fish solubles, 50% solids	2.50	50
Dried whey-product, 50% lactose	2.50	50
DL-Methionine	0.05	1
Calcium carbonate (or limestone), 38% calcium	1.60	32
Dicalcium phosphate, 20% phosphorus, 24% calcium	1.75	35
Trace mineral mix (Delamix)	0.10	2
Salt	0.50	10
Vitamin A, stabilized, 4000 I.U./g.	0.20	4
Vitamin D ₃ , 1500 I.U./g.	0.05	1
Choline chloride	0.07	1.4
Total	100.00	2000.0
Riboflavin	150 mg.	3 g.
Calcium pantothenate	250 mg.	5 g.
Niacin	1.5 g.	30 g.
Vitamin B ₁₂	0.3 mg.	6 mg.
α-Tocopheryl acetate	200 mg.	4 g.
Vitamin K (Menadione)	100 mg.	2 g.
Procaine penicillin G	200 mg.	4 g.
Arsanilic acid	4.5 g.	90 g.
Calculated Average Analyses		
Crude protein, %	21.2 ^b	Niacin, mg./lb. 30.2
Fat, %	2.6	Pantothenic acid, mg./lb. 6.9
Crude fiber, %	2.5	Choline, mg./lb. 780
Calcium, %	1.18	Vitamin B ₁₂ , γ/lb. 5.5
Phosphorus, total, %	0.74	B ₁₂ added 3.0
Phosphorus, available, %	0.49	Methionine, % 0.45
Phosphorus, inorganic, %	0.38	Lysine, % 1.05
Vitamin A, I.U./lb.	5728	Tryptophan, % 0.23
From stabilized A	3628	Arginine, % 1.17
Vitamin D, I.C.U./lb.	340	Productive energy, Cal./lb. 902
Riboflavin, mg./lb.	3.14	Energy/protein ratio 42.5

^a Proposed by a committee of the Animal Nutrition Research Council (U.S.), *Feed Age* 6, 41 (May, 1956).

^b Based on corn containing 8.5% protein and soybean oil meal containing 51% protein.

TABLE IV
PERFORMANCE TEST BROILER FORMULA^a

Ingredient		Per cent
Ground yellow corn		55.5
Soybean oil meal, dehulled, 50% protein		25.3
Alfalfa leaf meal		2.0
Dried whey		2.0
Butyl fermentation solubles		2.0
Fish meal		4.0
Fish solubles		2.0
Animal fat, stabilized		3.0
Ground limestone		1.4
Dicalcium phosphate		2.0
Salt		0.5
Vitamin A, 10,000 U.S.P. units/g.		0.05
Vitamin D ₃ , 3000 I.C.U./g.		0.04
Penicillin, 2 g. procaine penicillin/lb.		0.1
B-Complex vitamin concentrate ^b		0.05
DL-Methionine		0.1
MnSO ₄ , 4 ounces per ton of feed		
Antioxidant—added according to manufacturer's recommendations		

Calculated Analyses ^c			
Protein, %	22.0	Choline, mg./lb.	570
Fat, %	6.0	Vitamin B ₁₂	+
Crude fiber, %	2.45	Methionine, %	0.53
Calcium, %	1.40	Lysine, %	1.11
Phosphorus, total, %	0.91	Tryptophan, %	0.23
Vitamin A, I.U./lb.	4700	Arginine, %	1.23
Vitamin D, I.C.U./lb.	545	Productive energy, Cal./lb.	964
Riboflavin, mg./lb.	2.5	Energy/protein ratio	43.8
Niacin, mg./lb.	19.0		
Pantothenic acid, mg./lb.	6.0		

^a From E. L. Stephenson, *Feed Age* 6, 39 (August, 1956).

^b Contains 2 g. riboflavin, 4 g. calcium pantothenate, 9 g. niacin, and 20 g. choline chloride per pound of concentrate.

^c No analyses are given in the original publication. These values have been calculated by the present author.

fect of fat and carbohydrates on protein utilization has long been recognized by workers in the field of nitrogen and protein metabolism. It is doubtful, however, if they fully realized the magnitude of this effect and the influence of energy on the quantitative requirements for certain nutrients, especially the amino acids. If, in the past, the poultry nutritionist was preoccupied with the recognition, isolation, and identification of individual nutrients to the exclusion of consideration of the

performance of the diet as a whole, including the effect of its energy content, he has now corrected the situation; the latter subject presently is one of wide interest and intense experimental activity.

(1) *Development of high-energy diets.* Scott, Singsen, and Matterson (20, 21) are credited with first drawing attention to the value of "high-energy, low-fiber diets" in improving growth rate and efficiency of feed utilization in growing chicks. By designing a diet (the Connecticut broiler ration) in which relatively high levels of animal protein sources, such as fish meal, meat and bone scraps, and liver meal (8, 8, and 3%, respectively) replaced a major part of the soybean oil meal, and, omitting wheat by-products, oats, and much of the alfalfa meal, they were able to include over 69% of yellow corn in their formula. Their results showed that such a diet, properly supplemented with vitamins and minerals, supported excellent growth, feed efficiency, and feathering and greatly outperformed the then-current broiler rations.

Since the work cited above there has been much interest in high-efficiency diets and a steady trend to diets of higher energy content not only for growing chicks but also for laying hens. Low-energy items such as wheat by-products and oats have been reduced in amount or eliminated from the formula, and in line with this trend the use of dehulled soybean oil meal (50% protein) has increased. But of especial importance in this regard has been the increasing use of fat in the diets not only of poultry but of other domestic animals. An illustration of this in the broiler ration is seen in the Arkansas diet (Table IV). The great excess supply of animal and vegetable fats and oils in the United States has stimulated this trend, and many workers have shown that fats are not only economical sources of energy but, in addition, improve palatability and appearance and reduce the nuisance of dust in the handling of feed (cf. 22).

In order to estimate better the total energy content of different diets, use has been made of the productive energy values determined by Fraps for poultry feed ingredients (23). These values are estimates of net energy based on experiments with growing chicks and are usually expressed as large calories (Calorie or kilocalorie) per pound of feedstuff. There has been some critical comment concerning Fraps' procedure and the values which he has determined for certain materials; also the relative merits of using estimates of energy content in terms of metabolizable energy rather than productive energy have been considered (24, 25). It is common practice at the moment, however, to use the latter in dealing with poultry rations, as is illustrated in the two diets shown in Tables III and IV. Thus, it is calculated that the first contains 902 and the second 964 Calories of productive energy per pound. The value for the added fat in the Arkansas diet is taken to be 2900 Calories per pound, in the light of the findings of Hill (26), rather than the figure of about 2100 given by Fraps (23). Many commercial diets are offered today with an energy content equal to, or greater than, that of the Arkansas formula, and experimental diets of even much greater caloric content have been studied. For

comparison with older diets it may be noted that Fraps reported an average value slightly over 800 Calories per pound for all-mash starter rations in use during the 1940's.

(2) *Effect of high-energy diets on food intake.* The decreasing food intake which results from the use of diets of increasing energy content has raised the question of the adequacy of such diets in other nutrients and the nutritional standards by which they should be formulated. There are many instances in which increases in dietary energy, especially when brought about by the addition of large amounts of fat, actually inhibited growth (cf. 27, 28) because of an insufficient intake of some critical nutrient. The supplementation of high-energy diets with vitamins and minerals has not presented much difficulty. Fisher *et al.* (29) were not able to differentiate between the phosphorus requirements of growing chicks on diets of different energy content, and it is easy, and customary, to supply generous levels of needed vitamins to the formula. But the problem of adjusting protein (amino acid) intake in relation to energy has been more difficult, especially in the light of many recent findings that a near-optimal ratio is highly important in terms of growth and economy of gains. In considering how best to define requirements for proteins and amino acids in terms of different energy levels it might be in order to consider present dietary standards and how they were arrived at.

(3) *Dietary standards for protein and amino acids.* Nutrient requirements for animals are stated in terms of per cent of diet or amount per pound of diet, the assumption being (implied if not expressed) that different diets will be consumed in approximately the same amount. This is now known to be far from the true situation. As will be discussed later, many dietary conditions affect the amount that animals, under ad libitum feeding, will consume. Not the least of these are nutrient balance, especially amino acid balance, and nutrient concentration. It is to be remembered that compilations such as the authoritative National Research Council (NRC) Nutrient Requirements for Poultry (30) are summaries of separate experiments in which a nutrient, present in suboptimal amount, was added to a diet in graded amounts until response ceased (usually determined as the maximum weight obtained but sometimes also involving feed efficiency). Clearly, the level at which response stopped was affected by the next limiting nutrient of the diet, and hence all estimates of requirements are influenced by the nutrient make-up of the diet which was used. (See also Chapter 7.)

An illustration can be found in the thorough review presented by Hill (31) of the great range of values which has been reported, or can be calculated from reports, in the literature on the requirements for methionine, for

methionine plus cystine, and for lysine. Among the approximately forty values listed dealing with the sulfur amino acids, the estimated methionine requirement ranged from 0.28 to 1.2% and that for methionine plus cystine from 0.45 to 1.5% of diet; among the twenty-five lysine values the range was from 0.25% (5% protein diet) to 1.56% (40% protein diet), but the majority fell between 0.85 and 1.3% of diet. Hill concluded that the earlier estimate of

TABLE V
ESSENTIAL AMINO ACID REQUIREMENTS FOR CHICKENS AND TURKEYS^a

Amino acid	Starting chicks % of ration	Starting poults % of ration	Laying chickens % of ration
Arginine	1.2	1.6	?
Lysine	0.9	1.5	0.50
Histidine	0.15	?	?
Methionine	0.8	0.87	0.53
or			
{ Methionine ^b	0.45	0.52	0.28
{ Cystine	0.35	0.35	0.25
Tryptophan	0.2	0.26	0.15
Glycine ^c	1.0	1.0	?
Phenylalanine	1.6	?	?
or			
{ Phenylalanine ^d	0.9	?	?
{ Tyrosine	0.7	?	?
Leucine	1.4	?	?
Isoleucine	0.6	0.84	?
Threonine	0.6	?	?
Valine	0.8	?	?
For protein level	20.0	28.0	15.0

^a These data are those shown in Nutrient Requirements for Poultry, *Natl. Research Council* (U.S.) *Publ.* 301 (Rev. 1954).
^b Cystine will replace methionine for chicks as long as the ration contains not less than 0.45% methionine.
^c The chick can synthesize glycine, but the synthesis does not proceed at a rate sufficient for maximum growth.
^d Tyrosine will replace phenylalanine for chicks as long as the ration contains not less than 0.9% phenylalanine.

Almquist (32) of 0.45% methionine was somewhat generous but that 0.80% total sulfur amino acids was a satisfactory estimate, and that the Almquist figure of 0.9% lysine was low, since their own studies would indicate between 1.0 and 1.3% of the diet. A more recent publication from Cornell (33), dealing *in extenso* with the lysine data, gives the value of 1.1%, but different estimates were obtained with two different diets.

In Table V are shown the essential amino acid requirements for chickens and turkeys in the NRC publication (30) which are essentially those of

Almquist (32). (See also Tables II and III, Chapter 2.) The amounts of the various amino acids are indicated as applying to diets with definite levels of protein. Early experiments (34-37) established this relation between protein level and requirements for lysine, methionine, and arginine, the amounts being essentially in direct proportion, and it became customary to express amino acid requirements as per cent of the protein of the diet. Later studies (38), however, indicated that at higher protein levels, although the required amounts of the amino acid increased with increase in protein, the increase became a successively decreasing percentage of the protein. (See also Chapter 7.) Seemingly, at lower levels of protein the amino acids are required in direct proportion to protein content, but at higher levels they are required in some lesser amount. As will be shown later, the energy content of the diet is of prime importance in the above relations.

c. Energy and Methionine Requirement

When synthetic DL-methionine became available commercially, many laboratory and large-scale field trials were carried out to assess its value in practical diets for growing chicks and turkeys. Many of these tests showed striking improvements resulting from its addition, either in improved growth or feed efficiency or both. In some, however, such as those reported by Hill (31), there was little or no response. There seemed to be little correlation between the level of sulfur amino acids in the diets used and response to added methionine; actually some of the best results were obtained when methionine was added to diets containing generous amounts of animal products, such as fish meal, and considered to be adequate by NRC standards (39, 40).

An important step forward in understanding this confusing situation was provided by Baldini and Rosenberg (41), who demonstrated convincingly that the methionine requirement of the growing chick, expressed as per cent of diet, was directly related to the energy content of the diet. They reasoned that, as the energy content increased, the bird would consume less feed and that, therefore, the methionine intake, if maintained at the same percentage level, would decrease. Thus, high-energy diets would need more methionine, percentagewise, to assure an adequate intake, and diets which were only slightly deficient at a lower energy level would become distinctly deficient at a higher energy level. From this viewpoint, methionine and, presumably, all other nutrient requirements cannot be stated accurately as a fixed per cent of the diet but must be related in some way to energy content.

A preliminary linear regression of methionine requirement on productive energy content was obtained by examining the data from a large number of experiments (their own and those of others) involving methionine supplementation of the diets of growing chicks. Both the methionine and energy contents of the diets were calculated, and the point for each diet was plotted

on a graph with methionine content as ordinate and energy content as abscissa. It was found that a straight line could be drawn by inspection separating those diets which had been improved by a methionine addition from those which had not.

This linear relationship was tested further in three separate experiments. The results so obtained were in reasonably good agreement with the line first drawn and therefore confirm the postulate that the methionine requirement expressed as a per cent of the diet is linearly related to the energy content. The constants given for the regression line by Baldini and Rosenberg were $Y = 0.000736X - 0.2269$, Y being the methionine requirement in per cent and X the energy content in Calories of productive energy per pound (see Fig. 2).

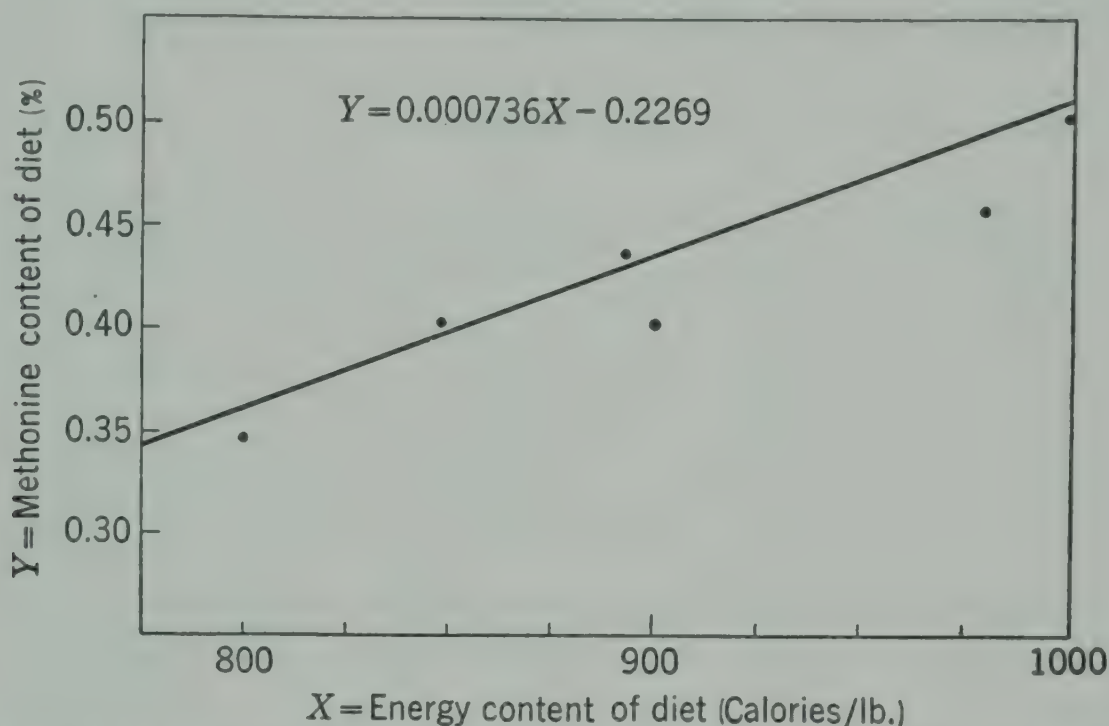


FIG. 2. Effect of dietary energy level on methionine requirement. The regression line was drawn first by inspection from assembled data. The points indicated represent estimates of requirement determined experimentally. In two of the three experiments the estimates of requirement coincided. From J. T. Baldini and H. R. Rosenberg, *Poultry Sci.* **34**, 1301 (1955).

It will be obvious that the data of Baldini and Rosenberg can be applied only to the limited energy range which they have studied, i.e., 800 to 1000 Calories per pound. It would be hazardous to extrapolate the values shown in the graph in either direction until the problem has been investigated further with diets of higher or lower energy content.

A preliminary report from the same laboratory (42) indicates that the response to added lysine in the diet of the growing rat is influenced also by the energy content of the diet.

These findings (which in retrospect seem so obvious) are of great interest in that they offer an explanation of some of the past results and give warning that the variable of dietary energy content will have to be controlled better. The first estimates of the methionine requirement of the growing chick by Almquist and his associates gave values ranging from 0.5 to 0.8%, which is understandable now, since they used rela-

tively high-energy diets (1000 Calories and over per pound) based on glucose, a single source of protein and, oftentimes, fat. On the other hand, the practical diets used in experiments reported by Hill (31), which were not improved by methionine addition, are known (personal communication) to have been relatively low in energy content (825 to 850 Calories per pound). In this connection the present NRC methionine requirement of 0.45%, using the regression line of Baldini and Rosenberg, would correspond to a dietary energy level of 920 Calories per pound. A question which immediately comes to mind is whether the values given as the requirements for the other essential amino acids are suitable for a similar level of dietary energy. Such a question can be answered only by further investigations which will relate the need for each of these amino acids to specific energy concentrations in the diet.

d. Energy Level and Protein Content. Calorie-Protein Ratio

It has been appreciated that, with high-efficiency diets, the traditional 20% of crude protein might not supply an adequate amount of the required amino acids for optimal performance. The suggestion of formulating diets for poultry in which the protein level would be in direct relation to energy content was first made by Combs and Romoser (43), and this additional method of describing a diet has come into common use. The relation is expressed as the total number of Calories of productive energy per pound for each per cent of crude protein in the diet and is referred to as the Calorie-protein ratio (C/P ratio). On the basis of several large-scale field trials and laboratory experiments (44, 45), these workers drew attention to the influence which varying C/P ratios have on body weight, efficiency of feed conversion, feather growth and feather pulling, and the fat content of the bird. They suggested a C/P ratio of approximately 42 (e.g., 1000 Calories per pound and 23.8% protein) for broiler starting rations to be fed for the first 6 weeks, and a ratio of 49 for the finishing ration to be fed after 6 weeks. The wider ratio during the finishing period recognizes the lessening need of the older bird for protein. It was realized that protein quality was of importance in establishing these ratios; this group attempted to obtain in their diets an "efficient" mixture of plant and animal proteins, including the addition of methionine, without, of course, having a recognized standard by which to judge the adequacy of the essential amino acids.

(1) *Effect of Calorie-protein ratio on methionine requirements.* Calculating the methionine level as a percentage of the protein in diets with different C/P ratios, these workers found an increased requirement for best performance as the ratio widened. It was suggested (45)

that for the C/P ratios of 42 and 49, which they recommended for practical broiler diets, a methionine level of 2.15% of the protein should be adequate. For wider ratios in either starting or finishing diets, the methionine requirement would be a larger percentage of the protein.

The difficulty of relating amino acid requirements to both energy and protein has been emphasized by Rosenberg and Baldini (46). They found, for example, that when protein was increased from 20 to 26% in a diet containing 1000 Calories, the increase in the methionine requirement was approximately proportional to the increase in protein level. But the same increase in protein in a diet of 900 Calories did not result in a proportional increase in methionine requirement. Thus the methionine requirement became a lesser percentage of the protein as the ratio of Calories to protein decreased (lower C/P ratio). They concluded that, if the energy level were sufficiently high to permit the protein to be used mainly for tissue synthesis, the amino acid requirements might be expected to be proportional to protein level. If, on the other hand, the non-protein energy level of the diet was low enough so that some of the protein was used for energy, then the amino acid requirements would not be proportional to the protein in the diet. It is interesting to note that the same suggestion was advanced in 1952 by Almquist (32) to explain why the requirements for lysine and sulfur amino acids became a lesser percentage of the protein as the latter was increased beyond normal levels. (See also Chapter 7.)

(2) *Effect of Calorie-protein ratio on body composition.* The attempt to define high-efficiency diets of differing protein and energy contents by using only gross body weight and feed efficiency as criteria yields results which are difficult of interpretation and are of significance only in comparing diets of quite similar composition. Fraps in 1943 (47) reported that the weight of 4-week-old chicks, the fat content of their bodies, and the amount of feed eaten were all influenced by the diet fed. The fat content of the carcasses of the chickens ranged from 1.4 to 16.3%, depending on whether he substituted cottonseed oil, protein concentrates, low-energy items, or combinations of these ingredients, for corn in the standard diet which he used. The protein content of the chickens, on the other hand, was fairly constant. The fattest birds were not those that consumed the greatest total number of calories but, in present-day terminology, they were the ones that received diets with the wider C/P ratios. This effect of different Calorie-protein ratios on body fat and moisture content has been noted also by Combs and Romoser (43). The studies of Hill and Dansky (48) and of Peterson *et al.* (49) show the same effect of diet variation on body composition but were done in a manner to allow some insight into the effect of die-

tary ingredients on voluntary feed intake which will be discussed further below.

The effect of dietary protein quality on the composition of body weight gains has been discussed by Allison (50). When growing puppies were fed diets with equal amounts of protein from wheat gluten or whole egg, there was no difference in weight gained per gram of nitrogen intake from either source. But very pronounced differences were found in the amounts of nitrogen retained in the bodies of the puppies. The puppies receiving egg protein were increasing their bodily protein stores much more rapidly than the puppies receiving wheat gluten; the

TABLE VI

THE EFFECT OF DIFFERENT PROTEIN AND ENERGY LEVELS ON GROWTH, FOOD INTAKE, AND FAT CONTENT OF DUCKS^a

Diet	Protein level	Fiber level	Energy level	Calorie-protein ratio	Average N.Y. dressed weight, 8 wk.	Average feed consumption, 8 wk.	Fat content, ^b 8 wk.
					lb.	lb.	%
Low protein-high energy	16.4	2.9	975	60	6.32	24.6	33.7
Low protein-low energy	16.0	6.3	835	52	6.29	26.1	29.4
High protein-high energy	18.4	3.4	940	51	6.36	23.8	30.0
High protein-low energy	18.4	6.2	790	43	6.11	26.4	26.2

^a These data taken from M. L. Scott, in "Poultry Comment," Vol. 13, E. I. duPont de Nemours & Co., Wilmington, Delaware, 1956.

^b Given as per cent of oven-ready carcass, fresh basis.

latter appeared to be in good physical condition but on closer examination were found to be soft and fat.

The practical application of differing ratios of Calories to protein in the diet in the production of a more acceptable carcass in market ducks has been described in a popular article by Scott (51). His findings are summarized in Table VI, which show that with Calorie-protein ratios ranging from 60 to 43 there was little difference in the average dressed weights of the carcasses. The differences in body fat content were found to be reflected mainly in the obvious subcutaneous and abdominal fat depots; the less fatty carcass was more acceptable to the consumer.

In further studies on the problem of formulating high-efficiency diets for poultry and non-ruminant farm animals, advancement will

come only through the gathering of data which will give more information as to the effect of the diet on the different tissues of the animal body. It would seem that both nitrogen and energy balances would need to be obtained for precise information.

e. Dietary Protein and Food Intake

It is stated generally that the need for energy is the most important stimulus to food intake and that, under conditions of ad libitum feeding, this requirement controls the amount of food eaten. There is evidence, however, that the need for protein of a definite amino acid make-up is also an important factor, less well appreciated. The profound effect on appetite of the absence from the diet of an essential amino acid was demonstrated by Frazier *et al.* (52). Animals will voluntarily consume widely varying amounts of energy depending on the quantity and quality of the protein in the diet.

Hill and Dansky (48), in studying the energy requirements of growing chicks, diluted a high-energy diet with finely ground oat hulls in two ways: (1) the oat hulls were *substituted* in 10% increments for corn and wheat with adjustment of the protein concentrate to maintain the crude protein at 20% of the diet (diminishing C/P ratio); (2) the oat hulls were used in similar increments to *dilute* the whole diet (constant C/P ratio). The energy content of the diets in both series decreased at approximately the same rate, but in the substitution series the proportion of the calories contributed by protein increased whereas in the dilution series the ratio of protein to non-protein calories was constant. Chicks receiving the diets containing oat hulls progressively increased their intake of feed in both series as the level of hulls increased up to 40% but not sufficiently to maintain their caloric intake, which became progressively less. In the substitution series the chicks were able to maintain a body weight equal to those on the basal diet even at the highest level of hulls, but in the dilution series they were not able to do so. The point of main interest here, and one commented on by the authors, is that in the substitution series those chicks on all the intermediate levels of oat hulls did not consume as much feed as they were physically capable of doing. There was a difference in total voluntary consumption of energy as the proportion of calories in the form of protein increased. A later report (53) from the same laboratory on further work of the same nature indicates that this is now interpreted as due to an effect on appetite.

The results of Williams and Grau (54) may be interpreted also as showing an effect of the proportion of calories in the form of protein on the voluntary consumption of total calories. These authors used a semipurified diet containing 15% of sesame meal supplemented with varying amounts of lysine. The substitution of cellulose for glucose in the diets resulted in improved growth on all the diets deficient in lysine and also increased the efficiency of the calories consumed (gain in weight per Calorie consumed).

The studies reported by Allison (50), and mentioned previously, are particularly revealing as to the effect of quality of protein on the voluntary food intake of growing dogs. A group of puppies was fed a diet containing

wheat gluten, and after 40 days some of the animals were changed to a diet containing an equivalent amount of defatted whole egg. The latter animals immediately decreased their daily caloric intake as compared with those on the wheat gluten diet. This decrease in food intake was reflected by a temporary dip in the growth curve, but this loss in weight was shown not to be due to a decrease in nitrogen content; balance studies showed that with the shift to good protein there was an increased storage of nitrogen.

Apparently, there is a physiological control involving both calories and amino acids; food intake is affected by the need for both and by the ratio in which they are supplied in the diet. The problem of variable food intake and its effect on body weight gains and the interpretation of nutritional experiments, particularly those dealing with amino acid supplementation, is more critical than is generally appreciated. It is discussed further in later sections.

2. Supplementation of Turkey Diets

The basic problems in the amino acid supplementation of turkey diets containing soybean oil meal as the main source of protein are essentially the same as those of chick diets. In view of the previous discussion only the special problems of meeting the nutritional requirements of the turkey will be dealt with. Knowledge concerning the latter is less precise than in the case of the chick, mainly because the poult is a more difficult experimental subject for reasons of size and less easy availability.

The turkey poult has the capacity for very rapid growth, and its known nutritional requirements expressed as per cent, or amount per pound, of diet are greater than those of the chick. The estimated requirements for protein and for those amino acids which have been investigated are shown in Table V. The observations on which these values are based have been reviewed by Almquist (32). As pointed out by Scott (55), some of the estimations of amino acid requirements were carried out with diets of protein content lower than 28% and have been calculated to the latter level on the assumption that requirements are in direct proportion to protein. Indeed, in the absence of direct experimental evidence as to the requirements for certain amino acids, approximations are obtained on the assumption that the poult requires 1.4 times the known chick requirements.

Regardless of the accuracy of the values for individual amino acids there is good evidence that the growing poult, for best performance, needs a high level of protein in the diet; recommendations have ranged from 24 to as high as 32%. There is also a high lysine requirement which is needed not only for growth but also for normal feather pigment formation in the bronze turkey. The occurrence of a white bar in

the growing feather was shown to be due specifically to a deficiency of lysine (56, 57). The appearance of this defect in pigment formation has aided in detecting borderline lysine deficiency; it appears that the growth of body tissue has a higher priority for the available lysine. The requirements for both protein and lysine decrease with age; normal feather pigment formation may occur spontaneously if the poult is continued on the diet which earlier produced this evidence of lysine deficiency.

The value of adding synthetic DL-methionine to the diets of growing turkeys in which the major portion of the protein was supplied by soybean oil meal has been demonstrated by many investigators (58-62). In some of these studies the cereal portion of the diet has included

TABLE VII

GROWTH AND EFFICIENCY OF JERSEY BUFF POULTS ON 20% AND 28% PROTEIN DIETS^a

Treatment	6-week gain	Gain/feed consumption	Gain/protein consumption
	g.		
20% Protein	671	0.435	2.17
20% Protein + methionine + lysine	807	0.461	2.27
28% Protein	764	0.431	1.54
28% Protein + methionine	812	0.440	1.56

^a These data were taken from J. T. Baldini, H. R. Rosenberg, and J. Waddell, *Poultry Sci.* **33**, 539 (1954).

milo, wheat, or oats in addition to corn. The amounts of methionine used have ranged from 0.025 to 0.2% of the diet, and improvement in growth or feed efficiency, or both, has been reported. Most of the observations were made during the early weeks of the poult's life, but several were continued until the birds were 24 weeks old. In one experiment (62) no advantage was gained from the addition of methionine to the diet after 8 weeks; in another (61) continued response to methionine was noted throughout the 24-week experiment.

The thought that the requirement of the young turkey for high levels of dietary protein might be due to the specific need for lysine led Baldini *et al.* (63) to test the efficiency of lysine and methionine as supplements to a low-protein diet. In three experiments they compared corn-soybean meal diets containing 20 and 28% protein, respectively, and found that the lower protein diet supplemented with both lysine and methionine supported as good growth and efficiency of feed conversion as the higher protein diet even when the latter was supplemented with methionine. The gist of their findings is shown in Table VII. These findings have been confirmed by others (64, 65); if syn-

thetic lysine were available in quantity and at a price comparable with methionine, there would be great commercial interest in its use, especially for the diet of poults during the first 6 weeks of life.

3. Supplementation of Laying Diets

The estimated protein and amino acid requirements (so far as the latter are known) for laying chickens are shown in Table V (30, 32). At protein levels of 15 to 16%, diets based on corn (or other cereal) and soybean oil meal have an amino acid pattern influenced as much by the cereal protein as by the soybean protein. The lysine content of the protein mixture decreases decidedly as the proportion of soybean meal in the diet decreases (see Fig. 1), but at such protein levels there seems to be no marked deficiency of any of the amino acids for which we have knowledge about their requirements for laying hens.

Mehring *et al.* (66) studied the effect of the addition of vitamin B₁₂ and of methionine on the hatchability of eggs produced by New Hampshire pullets in batteries receiving a corn-soybean-alfalfa diet supplemented with minerals and vitamins, except that no source of vitamin B₁₂ was included. The diet contained 16.8% crude protein and an estimated 0.25 to 0.31% methionine content. It was reported that the supplements, either singly or together, had no significant effect on egg production or feed efficiency of production but both had a beneficial effect on hatchability. The data indicated that the effects of vitamin B₁₂ and methionine were independent and additive. Heywang (67), using essentially the same diet for White Leghorn pullets on litter, found a supplement of B₁₂ to increase hatchability of the fertile eggs produced but no further increase when both methionine and B₁₂ were added.

As in other phases of poultry production, interest has been shown in the effect of varying dietary energy levels on rate and efficiency of egg production. The best-documented reports are those of Hill and associates (26, 68), who studied energy levels ranging from 740 to 1025 Calories of productive energy per pound of diet. They noted little effect on rate of production as energy was increased (except during the cold months of the year) but a direct relation between energy level and efficiency of production (weight of feed per unit weight of eggs); at the higher levels of dietary energy a constant protein level of 16 to 16.5% was maintained in the diets. In a further report they (69) concluded that 15% protein in a ration containing around 960 Calories of productive energy per pound was adequate to support maximum egg production. When a strain of Leghorns with a smaller mature body weight was used, the protein requirement was estimated to be between 15 and 16.5%, but the substitution of 5% fat in the ration did not seem to increase protein requirements. A "protein sparing effect" of fat was postulated.

The physiological mechanisms involved in the latter effect are not clear, but it suggests that at lower energy levels protein is being "wasted" in some manner. More precise information on the amino acids and the amounts of each required for optimal egg production at high energy levels would be of great interest. The amount of feed consumed by laying hens is a large proportion of the total poultry feed produced, and it is possible that supplementation of such diets with the amino acid in least supply would result in the saving of protein and a more economical dietary formulation.

Feather pulling and cannibalism are vices which develop frequently in laying flocks. Neal (70) has reported that he could suppress cannibalism and greatly reduce the number of pickouts by the direct addition of DL-methionine to the commercial ration which was being used. It was estimated that the ration contained 0.2% methionine and 0.25% cystine. The addition of 0.1% methionine was required to suppress completely the tendency to pick which Neal believes is an expression of methionine deficiency.

4. Supplementation of Swine Diets

Swine production is of great economic importance in the United States, particularly in the middle western states where corn and soybeans are main crops. It is reported (71) that 44% of total corn production is used in the feeding of hogs. Soybean oil meal has become, by far, the most important protein supplement to be used with corn in swine rations. These ingredients, in different proportions, plus certain known vitamins, minerals, and antibiotics seem to comprise a diet which will supply all the nutrients needed for growing swine. Small amounts of other ingredients may be included (alfalfa meal, animal products, wheat by-products, fermentation residues), but there is little evidence that they must be present to supply unidentified nutritional factors. Estimates of the essential amino acid requirements of the weanling pig are listed in the publication *Nutrient Requirements for Swine* (72) (see also Chapter 2), but some of the values given are of limited significance in the formulation of corn-soybean meal diets. The same comments apply here as were made in the discussion of amino acid requirements for growing chickens—all estimates of requirements are influenced by the type of diet given. The values listed were obtained with diets of widely varying energy and protein content.

Research findings during recent years (73–75) have been interpreted as showing that protein levels in corn-soybean meal diets, lower than previously used, would produce satisfactory growth and efficiency of feed conversion in growing fattening pigs. Protein levels of 14, 12,

and 10% of the diet are recommended, respectively, for the weight ranges of 50 to 100, 100 to 150, and 150 to 200 lb. live weight. For pigs of lesser weight than 50 lb. higher protein seems to be required, and this is particularly true of the pig weaned very early (5 to 8 lb. body weight) where at least 24% protein is needed.

a. Effect of Supplementation with Lysine and Methionine

Several investigators (76-78) have tested the supplemental value of lysine and methionine, singly or together, in rations of 12 to 16% protein for weanling pigs with evidence of response to one or the other of the added amino acids. Thus Catron *et al.* (77) noted a response in both growth and efficiency of feed utilization to 0.1% lysine added to a 12% protein diet with young pigs, but little or no response from its addition to a 14% diet. Methionine at 0.025 or 0.05% of the diet increased gains slightly on a 14% protein diet but had no effect on the 12% protein diet. Pfander and Tribble (76) also reported responses to low levels of lysine, methionine, or tryptophan with weanling pigs receiving diets of 14 to 18% protein containing corn, soybean meal, tankage, and wheat shorts. The above improvements were of questionable economic value and were no more than might be obtained by increasing the protein level. Meade (79), using pigs weighing 70 to over 100 lb. fed a constant amount of a corn-soybean diet of 15.9% crude protein content, found that nitrogen retention was not improved by supplements of tryptophan, lysine, or methionine, each amino acid being tested in the presence of the other two. The results suggest that, at protein levels of 12 to 15%, corn-soybean meal mixtures may be of borderline adequacy for both methionine and lysine and perhaps also for tryptophan. Under present methods of assessing diet performance it is difficult to demonstrate the true state of affairs.

b. Production of Lean Carcass

The production of overly fat carcasses has been a constant problem in swine production, especially for those produced on diets containing high-energy cereals such as corn or wheat. Many studies have been made with the object of producing a leaner carcass by some modified method of feeding. Recent reports (80, 81) indicate that this can be done by restricted feeding or by substitution in the diet of fibrous materials of low nutritive value but only at the expense of greatly reduced gain in weight and in some cases lower dressing percentages. In the light of the previous discussion one wonders if the problem cannot be approached more logically from a consideration of the balance between protein and energy in the diet.

Some bearing on this problem comes from experiments with corn-soybean meal diets, ranging in protein content from 10 to 20%, which were fed to pigs over the whole growth period of approximately 35 to 200 lb. live weight (74, 82). If it is assumed that the corn and soybean meal components in each diet totaled 96% (exact formulas not given), it may be calculated (cf. Fig. 1) that the 10% protein diet contained about 1080 Calories of productive energy per pound* and a Calorie-protein ratio of 108. The 20% protein diet contained only about 916 Calories per pound and a Calorie-protein ratio of 46; the other diets fell in between. All the measurements made on the slaughtered animals indicated that a leaner, more acceptable carcass was produced by the higher protein diets, although it was concluded that average daily gains and feed efficiency reached a maximum between 16 and 18% protein. Gross weight, of course, did not distinguish between lean and fatty tissue in the body of the pig. Actually, there was a suggestion in the data that the 20% protein diet was the more efficient during the earlier part of the growing period (initial to 75 lb.).

The experiments of Lassiter *et al.* (83) are of interest in this same connection. They studied nitrogen retention, using both small pigs (40 to 50 lb.) and large pigs (around 150 lb.), on diets containing varying amounts of corn and a protein supplement to yield protein levels of 10, 14, 18, and 22%. These diets also covered a wide range in total energy content (calculated as 1080 to less than 900 Calories of productive energy per pound) and in Calorie-protein ratio (108 to 41). Equal amounts of diet were fed to each pig during the preliminary and collection periods (2 lb. per day for the small pigs and 4.5 lb. per day for the large pigs). Nitrogen retention increased with increasing protein up to 18% with the pigs of both weights, although the authors report that the differences with the large pigs were not statistically significant. In view of the differences between the diets in total caloric content and in the proportion of protein to non-protein calories, it might be suggested that the animals on the high protein-low calorie diet were using protein as a source of energy and, hence, were unable to retain the nitrogen for the formation of new tissues. Isocaloric diets would suggest themselves as more suitable for such studies.

The results from both above-mentioned groups suggest that the whole problem of protein level in relation to energy content in corn-soybean diets for growing pigs should be reinvestigated.

The higher protein diets in these experiments are similar to those used for the growing chick; there is reason to believe that, with the increasing proportion of soybean protein, some deficiency of methionine might exist, thus limiting both growth and feed efficiency. Certainly the approach of including higher levels of well-balanced protein in the diet should be explored as a means of producing leaner carcasses. Examination of some of the results of experiments (80) in which fibrous material was substituted in the diet toward the end of the growing period shows that one main effect was to increase total protein

* It is appreciated that productive energy values determined by growing chicks may not be fully applicable to the present case, but they will serve to illustrate the point.

intake. This together with the reduced total energy of the diet resulted in a distinct shift in the ratio of energy to protein in the feed consumed. The swine nutritionist should undertake to define more exactly the effect of different ratios and particularly the effect of well-balanced protein in producing a leaner carcass without undue sacrifice of rate of gain or economy of production. The problem seems to be the same as that discussed earlier concerning the effect of diet on growth, feed efficiency, voluntary food intake, and body composition of growing chickens.

c. Diets for the Young Pig

The formulation of diets capable of nourishing the very young pig has received attention in several laboratories. Whether designed as the sole diet of early-weaned pigs or as creep feed to supplement sow's milk, they all contain high levels of protein and energy. Protein sources such as dried skim milk, casein, or isolated soybean protein have been used with only limited reliance on cheaper plant or animal materials. The finding (84) that the addition of a proteolytic enzyme, pepsin in particular, to such diets aids the young pig in the digestion and utilization of the nutrients suggests that this phase of swine production may be made more practical and economical. It may well be that, as more accurate information is acquired concerning the amino acid requirements of the young pig, the use of synthetic DL-methionine may aid in arriving at more effective and economical formulations.

IV. SUPPLEMENTATION OF DIETS CONTAINING COTTONSEED MEAL

The importance of cottonseed meal as a protein source in the economy of the United States, the effect of different methods of processing on the nutritional quality of the meal, and a description of meals suitable for use in the diets of poultry and swine are dealt with in detail in other chapters of this book. Our chief concern here is a consideration of the amino acid deficiencies of diets which contain protein derived, in the main, from cottonseed meal and the practical means by which these deficiencies may be made good.

If the values for the essential amino acids contained in cottonseed protein, as determined by chemical or microbiological analysis, are compared with those of whole egg protein or judged by the standard of meeting the requirements of growing chicks, the primary deficiency would appear to be methionine (see Tables I and II). By biological assay with laboratory animals, however, the primary deficiency is shown to be lysine. This anomaly is best explained by findings, such as

those discussed by Lyman *et al.* (85), which indicate that the lysine in many samples of cottonseed meal is only partially available to the rat or chick because of the formation during processing of protein-gossypol (or lysine-gossypol), or other protein complexes which are resistant to digestion. These workers found distinct variations in the protein quality of meals processed under different conditions and were able to correlate these variations with the total or bound gossypol content and also with the amount of response shown by growing chicks when lysine was added to the different samples of cottonseed meal. (See also Chapter 17.)

There is good agreement that the primary deficiency of corn- (or milo-) cottonseed meal diets is lysine, as shown by growth studies on chicks (86–88), turkeys (89, 90), and swine (91–93). The interpretation of some of the earlier studies is difficult because of dietary deficiencies other than those inherent in the amino acid pattern of the protein and because not all samples of cottonseed meal were low enough in free gossypol to be satisfactory for poultry and swine. The occurrence of dermatitis confused the results of some of the swine experiments. There were several observations indicating that the addition of lysine to diets containing cottonseed meal did not produce optimal results, or did not equal the performance of similar diets containing soybean oil meal.

The methionine content of cottonseed and soybean proteins is similar (5, 94, 119), and it is the second limiting amino acid of the protein of corn-cottonseed meal mixtures. This secondary deficiency was not noted in certain studies because it was obscured either by the more serious lack of lysine or some other less obvious nutritional deficiency. Stephenson, however, has reported results (19) which demonstrate well a basic principle of amino acid supplementation; i.e., the primary deficiency must be remedied before a secondary deficiency can be demonstrated and studied. In an experiment with growing chicks on a corn-cottonseed meal diet there was no response to the addition of either of three levels of DL-methionine; there was, however, a graded response in growth and efficiency of feed conversion to the addition of four levels of DL-lysine·HCl (equivalent to 0.04 to 0.16% L-lysine), indicating that this was the primary amino acid deficiency. When methionine was added together with the lysine there was then obtained a response to the former and, as the level of lysine increased, the level of methionine at which the best performance occurred also increased. These results show not only that lysine is the first and methionine the second limiting amino acid in corn-cottonseed meal diets but also that the quantitative relationships may be demonstrated under proper conditions.

The practical approach to the use of cottonseed meal in poultry and swine rations has been, of course, to combine it in varying proportions with soybean oil meal, the latter being a relatively good source of lysine. Recent observations (95, 96) suggest that a substantial portion of the soybean oil meal supplement in well-fortified rations for both growing chicks and poults may be replaced with good-quality cottonseed meal without adverse effect on growth or feed efficiency. The extent of the substitution of cottonseed for soybean meal will be influenced by the make-up of the diet, particularly by the level of protein and balance of amino acids in relation to energy content. Diets containing a mixture of soybean and cottonseed meals are still potentially deficient in methionine, but this has not been demonstrated experimentally.

An important economic consideration emerges from these studies: as long as soybean oil meal is relatively easily available for mixing with cottonseed meal in diets for poultry and swine, synthetic lysine would need to be quite low in price to be considered commercially for the supplementation of such diets.

V. THE AMINO ACID SUPPLEMENTATION OF CEREAL DIETS

The nutritive value of the proteins of cereal grains and their specific amino acid deficiencies long have been of interest because they make up such a large part of both human and animal diets. All the cereals are characterized by being relatively high in energy and low in protein content; diets consisting primarily of cereals have this characteristically large energy-to-protein ratio, which, as will be discussed presently, poses a specific nutritional problem. It is a sobering thought that in coming to consider nutritional improvement of cereal diets one immediately thinks in terms of human diets; such diets are known to be too low in protein for economical animal production. Previous discussion has dealt with mixtures of cereals and protein concentrates in which the latter have influenced the amino acid pattern of the diet as much as, if not more than, the cereal protein. Cereals, especially wheat and rice, constitute the bulk of the human dietary in many areas of the world; it is of interest to review briefly the studies which have been carried out to determine how they may be improved by amino acid addition and the problems involved in so doing.

1. Wheat

As a result of the classical studies of Osborne and Mendel (97) it was established that lysine was an important amino acid deficiency of wheat proteins. Since then it has been shown by various investigations involving rat growth (98-103), as well as nitrogen balance in adult

dogs (50) and protein-depleted human adults (104), that lysine is the primary amino acid deficiency of whole wheat, wheat flour, or wheat gluten. This is confirmed by analytical values for the essential amino acids of wheat protein in comparison with those of an "ideal" protein such as that of whole egg (4) (see Table I). All the above-mentioned experimental studies are in agreement in showing that the addition of lysine to wheat protein results in a marked improvement in the quality of the protein either for promoting growth or increasing nitrogen retention. In the latter regard, lysine-supplemented wheat gluten exhibited a nitrogen balance index equal to that of casein (50, 104).

Rosenberg *et al.* (99, 100) studied the growth obtained in weanling rats in a series of experiments in which increasing levels of lysine were added to a diet containing 90% dried commercial white bread supplemented with fat, minerals, and vitamins (including vitamin B₁₂). This basal diet contained 12.5% protein ($N \times 6.25$) and, by microbiological analysis, about 0.3% lysine. Additions of DL-lysine·HCl equivalent to levels of L-lysine ranging from 0.1 to 0.8% were studied, and with each increment up to 0.4 or 0.5% L-lysine there was noted an increased growth response in the young rats and a marked increase in food efficiency; higher levels produced a plateau in the curve of response. The authors reported that, if sufficient lysine were added to this bread diet, growth was obtained equivalent to that produced by the stock colony diet (21.5% protein from both plant and animal sources), and on this basis they concluded that lysine was the only important amino acid deficiency in commercial bread containing 3% non-fat milk solids. This latter point was investigated in further experiments (100) by testing methionine, valine, and threonine as possible secondary amino acid deficiencies in the basal bread diet. None of these in combination with lysine improved on the results produced by lysine alone, and the same was found to be true when both valine and threonine were added along with lysine. Therefore, the original conclusion was reiterated, viz., that the only important amino acid deficiency of commercial white bread as measured by rat growth was lysine.

Hutchinson *et al.* (103) also noted the pronounced effect of small supplements of lysine to a diet based on dried white bread which was baked from a formula containing no added milk solids. The basal diet, adequately supplemented with vitamins and minerals, contained 13.4% protein and 0.25% lysine. Additional lysine in increments of 0.06% produced increased weight gains in male weanling rats during a 4-week experiment up to a total addition of 0.24%. The 0.30% addition indicated that the plateau for growth rate had been reached. These workers did not test the addition of amino acids other than lysine but commented on the excellent rate of growth which lysine alone produced in their rats.

The experiments of Sure (101, 102) are not so clear-cut and are more difficult of interpretation. Diets were used in which ground whole wheat or wheat flour supplied all the protein (approximately 8 and 9% total protein, respectively), and the improvement in the growth of young rats due to the addition of lysine, valine, and threonine was studied. Sure found that, with both diets, lysine improved the rate of growth and protein efficiency, but the

further addition of either of the other two amino acids improved the performance still more, and the combination of all three amino acids produced the best results. The difference between his findings and those of Rosenberg *et al.* undoubtedly is due, basically, to the difference in protein content of the diets used in the two laboratories, but the true nutritional relationships involved remain to be worked out. In the meantime, the following comments may offer an explanation. In his study (102) on wheat flour diets, Sure chose 0.4% L-lysine (L-lysine·HCl?) as the only level at which this amino acid was fed. Considering the low protein content of the diet this amount may have been excessive, and it is entirely possible that lower levels might have produced better results. The effect of the further additions of valine and threonine (possibly also excessive in amount) may have been, in part, to counteract in some way the excessive amount of lysine. It has to be admitted, however, that when Sure limited certain groups of rats to a fixed food intake (7 grams per day) those receiving the basal diet plus all three amino acids grew somewhat better and had better food efficiency than those groups receiving supplemental lysine or lysine plus valine. It remains to be determined, nevertheless, if the same increased performance could have been obtained with much lower levels of amino acids and, indeed, with the lysine alone at the proper level.

2. Rice

The most important publication on the amino acid supplementation of rice diets for the purposes of the present discussion is that of Pecora and Hundley (105). They used a diet containing 90% of ground, white polished rice as the only source of protein, together with fat, minerals, and vitamins. This resulted in a low-protein diet ranging from a calculated 5.3 to 6.7% depending on the nitrogen analysis of the different lots of rice which were used. All the ten amino acids listed as essential for the growing rat by Rose (106) were tested as supplements to the basal diet, singly and in combinations of two to six. No one of the amino acids improved the growth of weanling rats over that supported by the basal diet, and many of the additions depressed growth. This was true also of lysine which was indicated as being the most limiting amino acid in the basal diet.

In testing the addition of the other amino acids in combination with lysine it was discovered that only threonine had a marked supplementary effect. This combination of lysine and threonine seemed to be unique in improving the basal diet, and the further addition of all pairs of the remaining amino acids and many of the possible combinations of three and four of them did not add to the favorable effect of lysine and threonine. Only the addition of all the essential amino acids simultaneously to the basal diet (thereby increasing the nitrogen content of the diet to 1.73%) improved the rate of growth over that supported by the combination of lysine and threonine. Therefore, it was concluded that lysine and threonine were the most deficient essential amino acids in

rice and that they were about equally limiting for rat growth. These conclusions have been accepted widely.

It is now proposed to offer an alternative explanation of the above results, admitting that until the problem has been explored further experimentally it is speculative. Pecora and Hundley state: "Each essential amino acid was added to the experimental rice diets in the amount

TABLE VIII
PROPORTIONS AMONG THE ESSENTIAL AMINO ACIDS IN RICE DIET COMPARED WITH THOSE PROPOSED BY W. C. ROSE FOR GROWING RATS

	1	2	3	4	5
	Amino acid requirements according to Rose ^a	Proportionate requirements (tryptophan = 1)	Amino acids contained in rice diet ^b	Proportions among amino acids in rice diet (tryptophan = 1)	Rice proportions as per cent of proportionate requirements ^c
	%		%		%
Lysine	1.0	5.0	0.22	2.44	49
Histidine	0.4	2.0	0.10	1.11	56
Methionine	0.6 ^d	3.0 ^d	0.23	2.55	85
Tryptophan	0.2	1.0	0.09	1.00	100
Threonine	0.5	2.5	0.27	3.00	120
Phenylalanine	0.7	3.5	0.43	4.77	136
Valine	0.7	3.5	0.44	4.88	139
Leucine	0.8	4.0	0.53	5.88	147
Isoleucine	0.5	2.5	0.35	3.88	155
Arginine	0.2	1.0	0.49	5.44	544

^a W. C. Rose, *Science* **86**, 298 (1937). Column 2 is column 1 rearranged with tryptophan taken as 1.
^b Values in this column taken from L. J. Pecora and J. M. Hundley, *J. Nutrition* **44**, 101 (1951). Column 4 is column 3 rearranged with tryptophan taken as 1.
^c Column 4/column 2, multiplied by 100.
^d The value of 0.4% as the methionine requirement (proportion of 2.0) was proposed later by Rose's group.

suggested by Rose (106) for the growing rat, making appropriate allowance for inactive enantiomorphs." Actually, the lysine supplement consisted of 2% DL-lysine·HCl (equivalent to 0.8% L-lysine, not 1.0%). Considered from the standpoint of balancing the amino acids provided by the low level of protein in the diet, the supplemental amounts of all the amino acids were grossly excessive. An alternative method to the one used by Pecora and Hundley for determining which amino acids were deficient in their basal diet and one which will give a better estimate of the amount required is illustrated in Table VIII.

In making the calculations shown in Table VIII the underlying principle is the use of the proportionality relationships among the essential amino acids suggested by Rose's values, not the absolute amounts (which undoubtedly apply only to the diet and under the conditions used by him). These proportions (tryptophan = 1), shown in column 2 of the table, have been used by others (107) as a means of estimating the quality of various proteins. The values in column 3 of the table are the estimates, made by Pecora and Hundley, of the essential amino acid content of the 90% rice diet. The proportions among these latter values are shown in column 4, and, finally, these proportions are calculated as percentages of the Rose proportions and listed in column 5.

An inspection of the figures in this column shows that lysine, histidine, and methionine are indicated as being present in lesser proportions in the rice diet than the other essential amino acids; because of the presence of some cystine in the diet it could be assumed, tentatively, that the sulfur amino acid supply is adequate. This leaves lysine and histidine for consideration. It is appreciated fully that no great accuracy can be read into the values in column 5 because they are influenced by the errors in the estimate of the amino acid content of the diet and also of the amino acid requirements of the rat. But, if histidine is truly a second limiting amino acid at the indicated level, it would require only about 0.035% lysine addition to bring this amino acid up to the level of histidine, at which point the latter would prevent response to further additions of lysine. Also, it is easy to calculate that about 0.23% lysine and 0.08% histidine would bring them both up to the level of tryptophan.

The whole point of these calculations and of this line of reasoning is to suggest that the amino acid supplementation of low-protein diets, such as those used by Pecora and Hundley [and by Sure (102)], should be re-examined, using low starting levels and small increments to establish a response curve and the point at which plateauing occurs. Pecora and Hundley actually report that they observed as much response to one-fifth the amount of the combination of lysine and threonine (0.16% L-lysine plus 0.12% L-threonine) as to the amount they first used, but no test was made of either separately at the lower level.

If it is accepted that the lysine supplement to the rice diet was excessive and produced a so-called amino acid imbalance (see the following section), it could be speculated that the effect of added threonine was solely to overcome this imbalance. This would lead to the conclusion that threonine is not truly an amino acid deficiency of rice protein. Until the problem has been restudied, this may be accepted as a credi-

ble interpretation. In any event, it must be concluded that, at present, the specific amino acid deficiencies of rice protein are in question and the quantitative aspects of the deficiencies are not known.

3. Corn

It is well established that the first amino acid deficiency of corn protein is lysine and that the second is tryptophan. Mitchell and Smuts (98) were among the first to demonstrate this relationship by the rat growth method in which lysine and tryptophan, singly and together, were added to a diet in which all the protein, 8%, was contributed by corn (except for that in the yeast supplement). The addition of lysine alone improved growth, but the addition of tryptophan alone regularly depressed body weight gains. When the latter was added in the presence of lysine, however, a distinct increase in both body weight gains and body length resulted.

The findings of Sure (108), suggesting that threonine, added on top of lysine and tryptophan (or lysine, tryptophan, and methionine), further improves protein efficiency of a corn diet, are open to the same criticism that has been directed against its use in rice diets. The amino acid additions made by Sure to diets containing about 7.7% corn protein are seen to be almost as excessive as those employed by Pecora and Hundley (105). Therefore, it may be contended that the effect of the threonine supplement was to ameliorate, in some way, the untoward effects of oversupplementation with the other amino acids.

Essentially the same interpretation may be made of the work of Sauberlich *et al.* (109), who studied the difference in nutritive value between low-protein and high-protein corn samples. They added cystine and cystine plus methionine to the basal diets, apparently in the belief that corn protein is deficient in sulfur amino acids. They concluded that the low-protein corn was deficient in lysine, tryptophan, isoleucine, threonine, and valine, whereas the high-protein corn was deficient in only lysine and tryptophan.

The essential amino acid proportions of corn protein based on average analyses compared with the proportions required by the growing rat (107) would indicate that, aside from lysine and tryptophan, there are no clear-cut deficiencies. It seems fair to state that no biological tests of sufficient accuracy have been done to identify definitely the third limiting amino acid of corn protein. From the point of view of practicality and the economics of the situation, multiple amino acid additions, such as used by Sure (108), cannot be recommended.

VI. LOW-PROTEIN DIETS, AMINO ACID IMBALANCES, AND APPETITE

There has accumulated during recent years a considerable literature on the subject of amino acid imbalances, sometimes also referred to as

amino acid toxicity or amino acid antagonisms. These terms have been used to describe the untoward effects which have been noted when certain amino acid additions were made to low-protein diets fed, generally, to young growing rats. Special examples of the problem have been discussed above under cereal diets, but the studies have been extended in certain laboratories, by the use of other diets and a wide variety of supplements, to the general problem of the effect of an excess of one amino acid on the utilization of other amino acids. No attempt can be made to deal with these studies in detail; the reader will find a bibliography and reviews of the problem among the following references (110-113). Among these are reviews by Elvehjem and Harper in which they have interpreted their own findings, and those of others, as indicating that relatively small excesses of any of the indispensable amino acids are "toxic," that specific cases of antagonism exist between certain amino acids (leucine-isoleucine, isoleucine-valine, phenylalanine-threonine), and that, in general, the amino acid supplementation of diets involves certain hazards.

It is believed that much of the work in this field is complicated by possible misconceptions as to "requirements" for essential amino acids, that the interpretation of the results may be quite other than that which has been made, and, especially, that most of the experiments carried out probably have little significance for any practical program of supplementing human or animal diets with synthetic amino acids.

It is agreed that amino acid imbalances are observed mainly with low-protein diets; the phenomenon is difficult to demonstrate with diets containing normal amounts of good-quality protein, and the addition of such protein to unbalanced low-protein diets overcomes the untoward effects. The latter consist almost entirely of poor growth; no pathological condition has been demonstrated, although some workers have followed fat deposition in the liver as an additional criterion. During an experimental period of a few weeks the basal low-protein diet permits the accumulation of three to four times the amount of fat found in the livers of well-nourished young rats; hence the efficacy of certain supplements has been judged by their effect on the level of liver fat. It has been observed that as the rats were continued for longer experimental periods the retarding effect of certain amino acid supplements tended to disappear.

1. Calorie-Protein Ratio

It has not been appreciated, apparently, by the workers in this field that the low-protein diets which they use are also very-high-energy diets. As an example, the 9% casein diet used by one group (casein,

9.0%; sucrose, 81.5%; corn oil, 5.0%; salts, amino acids, vitamins) contains about 1320 Calories of productive energy per pound.* Considering that the diet contains around 7.6% protein, this gives a Calorie-protein ratio of about 174. The rice diet (105, 114), lower in caloric content but also lower in protein, has a similar ratio. These ratios are extremely high, and such diets undoubtedly pose the young growing animal a difficult nutritional problem by forcing it to consume energy in great excess in order to obtain much-needed protein (amino acids). One wonders if this is not the principal reason for the accumulation of fat in the liver and, indeed, a basic factor in the whole problem of lipotropism. From this viewpoint, any dietary change which lowers the ratio of energy to protein would be beneficial—increase in the quantity of protein (or improvement in the quality) or reduction in the caloric content. The lipotropic effect of increased protein has been recognized (115), and one is led to suggest that were the energy content of the diet reduced, as, for example, by substituting a non-nutritive material like cellulose for some of the high-energy items, an improvement in growth and a reduction in liver fat would follow. Actually, there is an incidental statement in one of the papers (110) indicating that growth on a diet containing deficient protein was improved when 5% cellulose was included in the diet. The results of diluting the diet with non-nutritive substances have been discussed more fully in earlier pages.

2. Excessive Supplementation with Amino Acids

Comment already has been made on the manner in which certain low-protein cereal diets have been supplemented with amino acids, suggesting that the amounts used were excessive, and one would wish to repeat this comment concerning the supplementation of the low-protein diets which have been used in the study of amino acid imbalances. The 9% casein basal diet, used in some of the studies, contained 0.3% added DL-methionine (116) and, later, both 0.3% DL-methionine and 0.1% DL-tryptophan (110, 117, 118). It is presumed that the methionine was added because of the belief that casein is somewhat deficient in sulfur amino acids, and it is stated (116) that tryptophan was added to improve growth, although there is little evidence that it did so. The effect of these additions on the amino acid pattern of the casein diet, using the method already applied to the rice diet (see Table VIII), is shown in Table IX. In column 1 are listed the proportions among the essential amino acids suggested by Rose's values (tryptophan = 1). Column 2 shows the amount of each essential amino acid contributed by the

* It is appreciated that productive energy values determined by growing chicks may not be fully applicable to the present case, but they will serve to illustrate the point.

TABLE IX

PROPORTIONS AMONG THE ESSENTIAL AMINO ACIDS IN CASEIN DIET,^a SUPPLEMENTED AND UNSUPPLEMENTED, COMPARED WITH THOSE PROPOSED BY W. C. ROSE

	1	2	3	4	5
	Proportionate amino acid requirements (tryptophan = 1) ^b	Amino acids in 9% casein diet ^c	Proportions among amino acids in casein diet, unsupplemented (tryptophan = 1)	Casein diet proportions as per cent proportionate requirements (column 1)	Casein diet proportions, after addition of 0.3% methionine and 0.1% tryptophan, as per cent of proportionate requirements (column 1)
		%		%	%
Lysine	5.0	0.65	6.5	130	65
Histidine	2.0	0.24	2.4	120	60
Methionine	3.0 ^d	0.27	2.7	90	95
Tryptophan	1.0	0.10	1.0	100	100
Threonine	2.5	0.34	3.4	136	68
Phenylalanine	3.5	0.48	4.8	137	69
Valine	3.5	0.59	5.9	169	84
Leucine	4.0	0.76	7.6	190	95
Isoleucine	2.5	0.57	5.7	228	114
Arginine	1.0	0.32	3.2	320	160
Cystine		0.03			
Tyrosine		0.49			

^a See C. A. Elvehjem and A. E. Harper, *J. Am. Med. Assoc.* **158**, 655 (1955); A. E. Harper, W. J. Monson, D. A. Benton, and C. A. Elvehjem, *J. Nutrition* **50**, 383 (1953); A. E. Harper, W. J. Monson, D. A. Benton, M. E. Winje, and C. A. Elvehjem, *J. Biol. Chem.* **206**, 151 (1953); M. E. Winje, A. E. Harper, D. A. Benton, R. E. Boldt, and C. A. Elvehjem, *J. Nutrition* **54**, 155 (1954).

^b See Table VIII for requirements on which proportions are based.

^c These values calculated from R. J. Block and D. Bolling, "The Amino Acid Composition of Proteins and Foods," 2nd ed., p. 490. Charles C Thomas, Springfield, Illinois, 1951.

^d See footnote d, Table VIII.

9% casein (calculated, 7.65% protein), using the values of Block and Bolling (119); and the proportions among these values are shown in column 3. These latter proportions are shown in column 4 as percentages of the Rose proportions.

An inspection of the figures in this column indicates methionine to be only slightly deficient and, in view of the presence of some cystine, perhaps even less than indicated. It can be calculated that the addition of about 0.03% methionine, one-tenth the amount actually used, would have brought the proportion of this amino acid up to equal that of

tryptophan; the addition of 0.3% leads to a value of 190 for methionine in column 4. The further addition of 0.1% DL-tryptophan to the basal diet yields the amino acid proportions shown in column 5. By this addition the amount of tryptophan in the diet is essentially doubled (if it is assumed that the D-stereoisomer is fully active, which is not completely correct), and, since this amino acid is the base for calculation, the net effect is to halve the proportions of all the other amino acids. It is seen that the proportions are now quite different from what they were before the addition of methionine and tryptophan to the basal diet, and several amino acids—lysine, histidine, threonine, phenylalanine, and valine—are indicated as being in deficient supply. Because of the presence of an appreciable amount of tyrosine in the diet, it could be that phenylalanine is more nearly adequate than the value shown would suggest.

Interestingly enough, the results of Harper *et al.* (116) show that a mixture of lysine, histidine, threonine, and valine added to the basal diet in the presence of methionine and tryptophan (their amino acid mixture 5) was the most effective supplement that they tested, as measured by growth of the rats and the reduction in liver fat. Other amino acid mixtures or single amino acid additions were much less effective. It is believed that these results lend support to the proportionality method as a preferred procedure for determining which amino acids, and for obtaining some estimate of how much of each, are needed to provide a supplement suitable to the level of the other amino acids in the diet. If this assumption is correct it must be concluded that, in most of the studies on amino acid imbalances and low-protein diets in general, the amino acid supplements have been excessive and often have created imbalances equally as great as, but distinctly different from, those they were presumed to correct. It seems fair to suggest that the results obtained in many of these studies have little relevance to the actual problem of improving a low-protein diet nutritionally.

3. Food Consumption

A brief discussion of the effect of dietary protein on voluntary food intake was presented earlier in this chapter. Variable food intake may offer an explanation, simpler than others that have been postulated (111, 112), for the variations in body weight gain and liver fat content that have been noted after the addition of deficient protein or high levels of specific amino acids to low-protein diets. It is understandable that the young growing animal, subsisting on a low- (or poor-) protein high-energy diet and being in poor nutritional status, might possess an appetite peculiarly sensitive to dietary change, particularly one which did not improve its nutritional lot. There is considerable evidence to

this effect, and several investigators have commented on the phenomenon.

Mitchell and Smuts (98), who used the paired feeding method and, therefore, were able to observe differences in the amounts voluntarily consumed by the animals on the supplemented and unsupplemented diets, commented on the "relation between appetite and dietary balance." Frazier *et al.* (52) noted that the absence of an essential amino acid from the amino acid mixture which they used caused a sharp decrease in food intake with consequent weight loss, and they speculated as to the mechanism governing the impairment of appetite under these conditions. Interestingly, when the diet containing the deficient amino acid mixture was given by gastrostomy tube, the animal still lost weight but less so than the control animal being offered the same diet *ad libitum*. The forced feeding of the unbalanced diet produced no obvious "toxicity."

The most direct evidence as to the effect of the level of an amino acid supplement on appetite is provided by the report of Hutchinson *et al.* (103). They added lysine in increments of 0.06% to a dried bread diet fed to weanling male rats and observed increased growth accompanied by increased average daily food consumption up to the level of 0.24% added lysine; the 0.3% addition indicated that a plateau had been reached in both growth rate and amount of food eaten. In a second experiment, to determine if a greater level of lysine would induce an amino acid imbalance, these workers compared the response to supplements of 0.3 and 2.1% lysine and found that both growth and food consumption were less on the higher level (the differences were more pronounced during the first week, progressively less during the second and third). They concluded that the reduced rate of gain could be accounted for wholly by the reduced intake of diet. A similar relation between level of lysine supplement added to a bread diet and amount of food eaten may be calculated from the food efficiency figures reported by Rosenberg and Rohdenburg (99).

Great variation in amount of diet consumed after various amino acid supplements is evident in several other publications already reviewed (101, 102, 105, 108, 109), and in the papers of Gessert and Phillips (120) and Sauberlich (121). Where body weight change is the main criterion, the net effect of a dietary addition will be the resultant of two variables—the amount of food eaten and the physiological effects of its nutrient content. It is difficult, therefore, under conditions of *ad libitum* feeding, to estimate the true value of a supplement fed at a single level, arbitrarily chosen. In any event, it must be concluded that the effects of amino acid imbalances are mediated in large degree through their influence on appetite. This raises an interesting question as to whether certain amino acids have a unique effect on appetite, especially in overcoming an excessive supplement of some other amino acid. Threonine has been shown by several workers to reverse imbalances due to other amino acids, and one wonders if this effect is

due to a true requirement for threonine or to a specific effect on appetite.

In many publications on amino acid imbalances, antagonisms, and liver fat accumulation, strangely, no data are given on food consumption, and one must assume that no thought was given to any effect which this uncontrolled variable may have had on the results which were obtained. One may speculate that certain additions to the basal diet may have depressed food consumption and that others may have increased it. Many supplements decreased liver fat but also decreased weight gain, and both effects could have resulted from lowered food intake if it is accepted that the caloric intake is a factor in determining the amount of fat found in the liver. Further, the conclusions that leucine is a specific antagonist of isoleucine (110) and that definite antagonisms exist between other amino acids (122) must be regarded with skepticism until an evaluation has been made of the influence that varying food intake might have had on the differences observed in body weight gains. These comments do not deny that metabolic interrelationships may exist between different amino acids (aside from their synthesis into tissue protein) and that an excess of one might influence the mode of utilization of others, but such interactions in experimental animals cannot be regarded as established on the basis of the limited approach which has been taken to such a complex problem.

4. Guiding Principle for Supplementation

Finally, it seems fair to state that the studies which have been reviewed in this section do not represent a practical approach to the problem of improving human or animal diets by supplements of amino acids. The imbalances which have been observed were precipitated by excessive supplementation due, apparently, to misconceptions as to amino acid requirements, and were accentuated in most cases by the high-energy diets used. *The guiding principle in the supplementation of any diet (or foodstuff) should be to add the most limiting amino acid only in the amount needed to bring the total into balance with the amount available of the second limiting amino acid; if it is decided to supplement also with the second limiting amino acid, then both the first and the second should be brought into balance with the third limiting amino acid.* It is believed that this approach is nutritionally sound and it will be found to be economically imperative. For practical purposes multiple additions generally will be found infeasible. It may be taken as axiomatic that low-protein diets will require very small amounts of supplement. Amino acid imbalances or antagonisms need not be a problem in any program of supplementation.

VII. CONCLUSION

In the preceding pages an attempt has been made to review the present status of synthetic amino acids and the work which has been done to determine their utility in the supplementation of various proteins. Greatest emphasis has been placed on a review and interpretation of recent animal experiments which have helped to define more accurately the dietary conditions under which amino acid supplements may be used most efficiently and economically. Although past research has established well the basic principles of amino acid nutrition, the quantitative aspects of providing them in the diet have been less well appreciated.

It is difficult for the writer to consider the use of synthetic amino acids other than as relatively small amounts of supplements to either complete diets or to single food items which constitute an important part of the diet. That chemical synthesis can compete with natural sources in supplying the bulk of the amino acids is most unlikely. This is in contrast with the belief, apparently entertained by some, that the development and production of synthetic amino acids will parallel that of the vitamins over the last quarter century and that it is only a matter of time until all the essential amino acids are available in quantity at a price permitting their wide use. This latter view seems highly unrealistic. That methods of synthesis exist for all the important amino acids is not doubted, but that they can be produced economically and in quantity or that we know how to utilize them is a different matter. The preparation of a tablet for human consumption containing the daily requirements of all the essential amino acids, a suggestion reported to have been made (6), could have value for experimental or limited clinical use, but any prospect that such a preparation might serve as a means of improving the nutrition of an important segment of the world's population would seem to be extremely remote. Compared with the need for vitamins, amino acids are required in quantity and, very importantly, must be consumed regularly and in combination with other nutrients, particularly an energy source, to serve a useful nutritional purpose.

From the standpoint of supplementing natural foods or food products it is significant, and perhaps insufficiently appreciated, that the first limiting amino acid of the majority of the important protein sources is either methionine or lysine; in some cases the one is the first and the other the second limiting amino acid. On the basis of present knowledge one cannot identify a third amino acid which might compare with these two in potential for improving the quality of the world's supply of protein. Until precise information is provided as to the next

important amino acid and the foods that would be improved by its use, there will be little commercial interest in developing an attractive synthesis and in providing plant and equipment for its large-scale production. In the meantime it would seem wise to explore more fully the benefits which may be obtained from a wider use of methionine and lysine.

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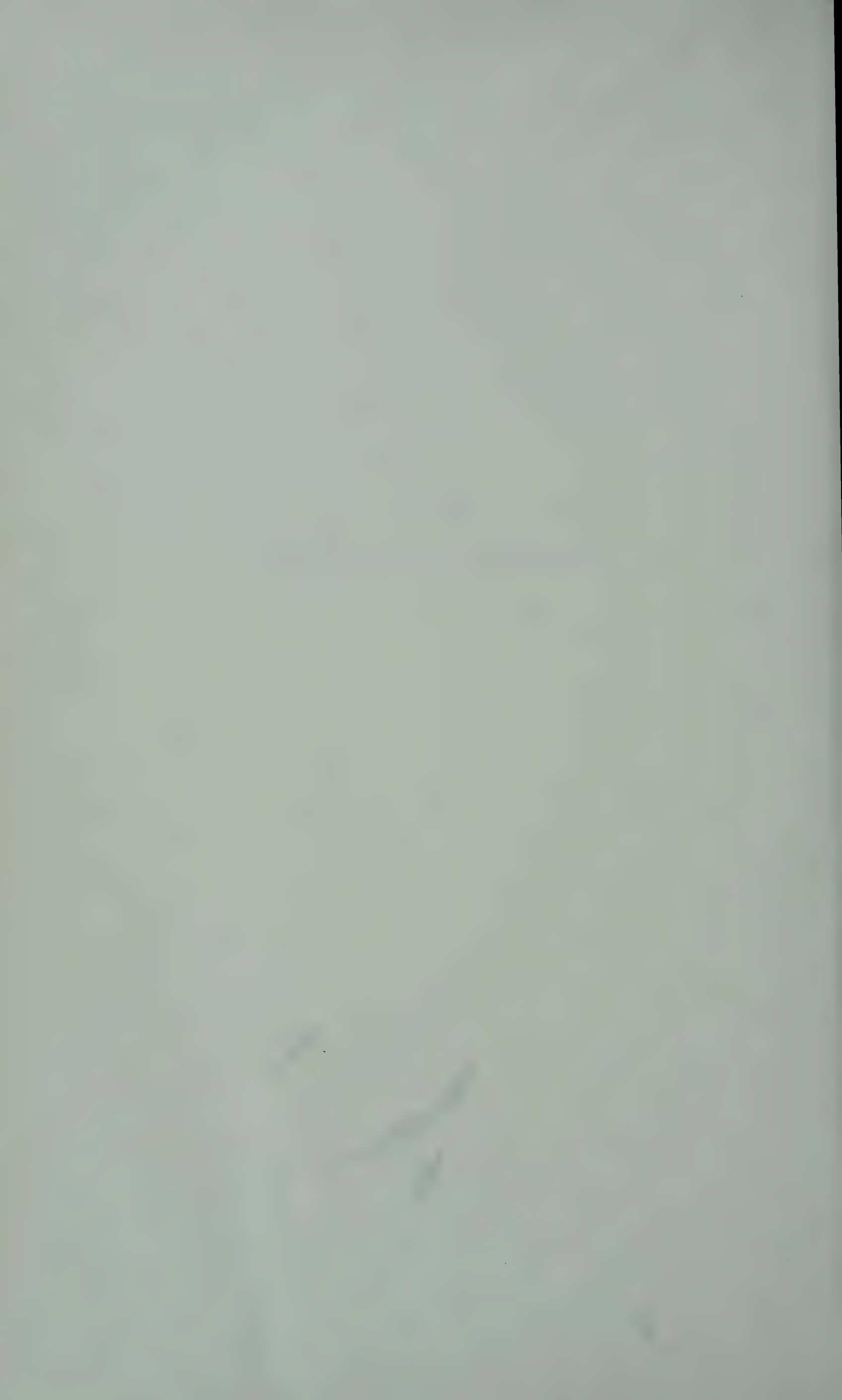
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PART II

PROCESSED PLANT PROTEINS



CHAPTER 14

SOYBEAN OIL MEAL

W. W. CRAVENS AND ENDRE SIPOS

I. INTRODUCTION

For centuries, soybeans and soybean products have constituted the chief source of protein for millions of oriental peoples. The soybean is native to Eastern Asia and plays a much greater role in the nutrition of the oriental people than does wheat in the United States, or rye in Germany, Scandinavia, and the Soviet Union. It was cultivated extensively and valued highly as a food centuries before written records were kept. Some of the first written records of the plant were in 2838 B.C., and the soybean is mentioned repeatedly in later records. In addition to the uses of the beans, many references were made in the ancient literature to the processes of preparation and the value of soybean sauce, soybean milk, soybean curd, soybean paste, and soybean sprouts as foods, and for the treatment of various diseases and body ailments. It is interesting that many of the known nutritional attributes of the soybean, proved by research in recent years, were known to the ancient Chinese people, although of course no scientific explanation could be given at that time.

No mention has been found of soybean oil in ancient Chinese literature, so it may be concluded that the crushing of soybeans for oil has occurred in comparatively recent times. The processing of soybeans, however, was more or less localized until after the Chinese-Japanese War (1894-1895), when Japan began to import soybean oil cake for fertilizing purposes, resulting in a sudden expansion of demand for this product. Soybean cake then became the chief end-product of the oil-meal industry. The Russo-Japanese War increased the production of soybeans in Manchuria, and, when this war ended, a surplus of soybeans developed. Japanese firms realized very soon the export potential of this crop, and in 1908 several shipments were made to Europe. After this time the soybean was one of the chief export items of this area.

Apparently the first soybean oil meal produced from American-grown soybeans was made by the screw-press process in 1915. The first mention of soybean production in the United States was in 1804, but serious introductions of the plant were not made until 1898, when the United States Department of Agriculture brought in several varieties. From this meager beginning at the turn of the century, the soybean has grown to be one of the most important crops produced in the United States.

The reader is referred to the writings of Markley and Goss, Morse, Bailey *et al.*, and Piper and Morse for a review of the history of soybean production (1-4).

II. PRODUCTION AND TRADE

1. General World Situation

Until 1940 the Orient (especially China and Manchuria) dominated the world markets for soybeans. In China proper the entire crop, which averaged around 200 million bushels in previous years, was used by the Chinese population as a major item in its food supply. In Manchuria, however, there was always a definite surplus for export. Exports went to other Asiatic countries before the Russo-Japanese war in 1904 and to all parts of the world thereafter. Manchurian soybean production reached its peak in 1930 (198 million bushels) (5) and declined sharply after that owing to the hostilities and Japanese occupation. The surplus exported by Manchuria amounted to more than 90 million bushels in the early 1930's.

In addition to the soybean trade, the strong demand from the United States for soybean oil during World War I induced the development of soybean processing plants in Manchuria which exported 350 million pounds of oil in 1926. With the development of European processing facilities, however, the demand for oil gradually decreased. After 1939, Japan and the Soviet Union were the only markets left for the oil.

Limited information indicates that soybean production in China-Manchuria increased considerably in 1954, the estimated crop being 350 million bushels (6). The prospect is still larger for 1955, for an effort is being made to ease the short supply of vegetable oils and regain part of the export market of prewar years. Soybean exports from China-Manchuria were estimated unofficially at 23 million bushels in 1953 (7).

Japan, Formosa, and Korea took about 85% of the Manchurian exports for soybean cake in 1929-33. The United States exported some

“beanboards” for livestock feed, but the major use was as fertilizer for the rice fields in Japan and for the sugar cane plantations in other parts of Asia.

Although soybeans are a substantial part of the Korean and Japanese agriculture, exports from Korea were never large and in Japan the crop supplied only a small fraction of domestic consumption. In both countries soybeans represent indispensable daily food. Since 1948 Japan has again become the major world market for soybeans; in 1953 over 40% of the total bean shipments from the United States went to Japan (8). Southeast Asia, the Indonesian Republics, and Formosa are also producers of soybeans, although there this crop does not have the same preeminence as in China, Manchuria, Korea, and Japan.

Successful attempts have been made to produce soybeans in many European countries, such as Italy, Bulgaria, Yugoslavia, Rumania, Germany, and Austria. Canada produces about the same amount as prewar Europe (5 million bushels). A substantial increase has occurred in South America (Brazil), and in certain parts of the U.S.S.R. soybean crops are now of great importance.

TABLE I

SOYBEANS: ESTIMATED PRODUCTION, WORLD AND MAJOR PRODUCING COUNTRIES^a

Country	Average, 1935-39	Annual		
		1952	1954	1955
<i>1000 long tons</i>				
Canada ^b	6	111	133	151
United States	1,504	7,983	9,149	9,944
China	5,562 ^c	5,357	4,821	{ 8,839 ^d
Manchuria	4,052 ^c	3,348	3,749	
Japan	3,304	513	370	487
Indonesia	260	259	390	401
Others	704	485	604	588
World total	15,386	18,056	19,216	20,410 ^e

^a U. S. Dept. Agr. Foreign Agr. Circ. FFO-5-56 (1956). Conversion factor: bushels to long tons based on $37\frac{1}{3}$ bushels per long ton or 0.026785 ton per bushel.

^b Average of less than 5 years.

^c Unofficial estimate.

^d China included with Manchuria.

^e World production (exclusive of U.S.S.R.) in 1956 is estimated at 23.8 million tons. United States production is estimated at 12.3 million tons. Food and Agr. Organization U.N., Monthly Bull. Agr. Economics and Statistics 6 (4), 10 (1957).

Germany encouraged the Danubian countries to grow soybeans during World War II by guaranteeing a specific price for contracted acreage. Production in Rumania, Bulgaria, and Hungary, which reached more than 6.5 million bushels per year, declined to less than 1 million bushels after the war.

The estimated production for the world and for the major soybean-producing countries is given in Table I.

2. United States

a. Production

There are three main areas of soybean production in the United States: (1) the North Central Region (Indiana, Illinois, Iowa, Ohio, Missouri, Minnesota, Kansas, Michigan, and Wisconsin); (2) the Mississippi Delta (Arkansas, Mississippi, and Louisiana); and (3) the Middle Atlantic Coast Region (North Carolina, Virginia, Maryland, and Delaware). The favorable soil and climatic conditions of the Corn Belt, the high yields and oil content of its soybeans, the local processing demand and livestock markets, as well as the unrestricted shipping potential of this area have led to its predominant position in the production of soybeans (9). The five principal Corn Belt States (Illinois, Indiana, Ohio, Iowa, and Missouri) accounted for 50% of the United States total production in 1929, and 70% in 1948. In 1954 these same states represented only approximately 61% of the total acreage owing to increased production in other parts of the country where soybean production previously had not been too successful. A good example is Minnesota, which, being on the fringe area of the Corn Belt, was one of the latest states to develop soybeans; yet today it ranks fourth in total production of the United States.

In addition to the rapid increase in acreage of soybeans (5.9 million in 1941, 11.4 million in 1947, and 17.0 million in 1954), the yield per acre increased from 11 bushels in 1924 to 19.4 in 1946-48, and it averaged 20.4 for the 1950-54 period.

Production in the Corn Belt States (90 million bushels) was 93% of the national total in 1939 and averaged around 85% (167 million bushels) in the years of 1942-48. The total United States production in 1955 was well over 300 million bushels, about four times that of 1939 (78 million bushels), and the Corn Belt States made up approximately 75% of the national total in both volume and in dollar value.

The total acreage, yield, and production in the six most important soybean producing states of the United States in 1954 were reported as follows (9):

State	Acres harvested for beans ^a	Average yield per acre harvested for beans	Total production for beans
	1,000 acres	bushels	1,000 bushels
Ohio	1,165	25.5	29,708
Indiana	1,922	24.0	46,128
Illinois	4,289	21.5	92,214
Minnesota	2,014	21.0	42,229
Iowa	2,150	26.0	55,900
Missouri	1,836	15.0	27,540

^a Equivalent solid acreage (acreage grown alone, with an allowance for acreage grown with other crops).

The acreage, yield, production, utilization, and value of the production in the United States are summarized in Table II.

b. Movement in Trade

In the United States, soybeans at first were grown primarily as a forage and pasture crop. With the adaptation of improved methods of culture, the development of markets for the oil, the technical advances in processing to enhance protein quality, and advances in nutritional knowledge making possible the utilization of soybean oil meal in feeding, the demand for seeds gradually increased. In 1939, 40% of the total acreage was harvested for seeds, 85% in 1947, and 93% in 1954.

Several factors helped the soybean industry grow in the United States. Corn acreage limitations in the late 1930's made soybeans compete effectively for the restricted acreage. The establishment of standards for grading and marketing by the U. S. Department of Agriculture as early as 1925, the establishment of a futures market for soybeans in Chicago in 1936, and the research activities of commercial and public institutions such as the Soybean Laboratory of the U. S. Department of Agriculture in Ohio, the U. S. Regional Soybean Laboratory at Urbana, Illinois, and the Northern Regional Research Laboratory of the U. S. Department of Agriculture at Peoria, Illinois, all served to stimulate soybean production.

Under these favorable conditions the United States in 1931 began to compete with Manchuria and by 1942 had surpassed Manchuria in production and processing of soybeans for oil and meal.

When war disrupted trade with Asia and the United States became the chief supplier of fats to the United Kingdom and the U.S.S.R., the demand for fats and oils was greatly increased. In 1942 the Government's appeal for increased soybean production to meet the wartime needs gave great impetus for both producers and processors. Programs for processors, which reduced their risks, and support prices for the farmer contributed to high production.

Improved feeding practices involving the use of soybean oil meal and increased animal products consumption as a result of higher living standards and

TABLE II
SOYBEANS: ACREAGE, YIELD, PRODUCTION, UTILIZATION, AND VALUE OF PRODUCTION
IN THE UNITED STATES,^a SELECTED YEARS

Year	Planted acres ^b (1,000 acres)	Acres harvested for beans (1,000 acres)	Average yield per acre for beans (bushels)	Total production for beans (1,000 bushels)	Processed for oil and meal ^c (1,000 bushels)	Exports ^c (1,000 bushels)	Farm value of production (1,000 dollars)
1929	2,807	708	13.3	9,438	1,666	—	17,736
1939	10,920	4,315	20.9	90,141	56,684	11,833	73,049
1942	14,912	9,894	19.0	187,524	133,454	697	301,137
1945	13,807	10,740	18.0	193,167	159,459	5,042	402,234
1947	13,755	11,411	16.3	186,451	161,397	2,985	621,477
1949	12,456	10,482	22.3	234,194	195,265	17,034	506,474
1950	15,744	13,814	21.7	299,279	251,990	26,904	737,822
1951	15,735	13,545	20.9	282,477	244,380	16,009	769,926
1952	16,420	14,338	20.8	298,052	234,404	30,392	809,314
1953	16,792	14,679	18.3	268,528	213,158	41,498	731,721
1954 ^d	19,253	17,037	20.1	342,795	249,000 ^e	43,547 ^e	863,596
1955 ^d	19,669	18,559	20.0	371,276	280,000 ^e	67,431 ^e	800,320 ^f
1956 ^d	20,953	—	22.4	470,064	—	—	—

^a "Agricultural Statistics," 1952 and 1955, Tables 189 and 180, respectively. U. S. Govt. Print. Off., Washington, D.C.
^b Equivalent solid acreage (acreage grown alone plus one-half the interplanted acreage).
^c "Agricultural Statistics," 1952 and 1955, Tables 197 and 186, respectively. U. S. Govt. Print. Off., Washington, D.C.
^d Preliminary.
^e U. S. Dept. Agr. Foreign Agr. Circ. FFO-5-56 (May 28, 1956).
^f Computed on the basis of average price for beans of \$2.16 per bushel.
^g Crop Estimates, October 1956. See also U. S. Dept. Agr. Foreign Agr. Circ. FFO-7-57 (June 12, 1957).

an increasing population has meant continued progress for soybeans to the present date.

c. Economic Importance of Soybean Products

(1) *Soybean oil meal.* Soybean oil meal (including pellets and cubes) for livestock feed is the major soybean product in the United States, as is shown in Table III. As a result of research and wartime experience, soybean oil meal gained wide acceptance in manufactured feeds, and, in the period of 1942–47, 90 to 95% of the total output was used in feeds.

The estimated use of soybean oil meal for animal feeds in the United States in 1947–48 was 54% of that of all oilseed meals for feeding and 34% of all high-protein feeds. Production of soybean oil meal

reached its peak of 5.9 million tons in 1950–51, of which almost 97% was used in feeds. Production then gradually decreased between the years 1951–52 and 1953–54. In the year 1953–54, total oilseed consumption, excluding soybean oil meal, amounted to 3.7 million tons; soybean oil meal in feeds was almost 5 million tons (10). This amount is equivalent to approximately 40% of all high-protein feeds. Production increased sharply again in the year 1954–55 to 5.7 million tons (11).

TABLE III

SOYBEANS: YIELD, PRICE, AND VALUE OF PRODUCTS PER BUSHEL OF SOYBEANS CRUSHED, UNITED STATES, 1947–54^a

Year begin- ning October	Oil			Meal			Value of products		
	Yield	Price ^b	Value	Yield ^c	Price ^b	Value	Total	Distribution	
	(pounds)	(cents)	(dollars)	(pounds)	(cents)	(dollars)	(dollars)	Oil (%)	Meal (%)
1947	9.5	23.7	2.25	47.5	4.04	1.92	4.17	54	46
1948	9.8	13.1	1.28	47.2	3.30	1.56	2.84	45	55
1949	9.9	12.3	1.22	48.0	3.22	1.55	2.77	44	56
1950	9.7	17.8	1.73	47.6	3.22	1.53	3.26	53	47
1951	10.0	11.3	1.13	47.8	4.17	1.99	3.12	36	64
1952	10.8	12.1	1.31	48.5	3.38	1.64	2.95	44	56
1953	11.0	13.5	1.48	48.4	3.93	1.90	3.38	44	56
1954 ^d	10.9	11.9	1.30	46.8	3.04	1.42	2.72	48	52

^a U. S. Dept. Agr., Agr. Marketing Service, Fats and Oils Situation, 1956 Outlook Issue, FOS-175 (Nov. 30, 1955).

^b Simple average price per pound based on the following quotations: soybean oil, crude, tank cars, f.o.b. midwest mills; soybean meal, bulk, Decatur, quoted as 41% prior to July 1950, 44% beginning July 1950.

^c Excludes beans crushed for flour; if these beans were included, the yield would be about 1 pound less.

^d Preliminary.

Virtually no soybean oil meal was imported during World War II and the postwar years. Exports have fluctuated greatly during the years 1939–1954, with no definite trend until 1951–52; thereafter there has been a steady increase in foreign trade, amounting to about 67 million tons in 1953–54.

(2) *Soybean flour and isolated and modified soya proteins* (5). Government orders for lend-lease exports during World War II and orders by the U. S. Army and by the United Nations Relief and Rehabilitation Administration for Europe and Asia increased production

to more than 300 thousand metric tons in the crop year 1947-48. Its usage for the civilian population of the United States did not prove popular, however. In the following year, 1948-49, domestic production decreased to 72 thousand tons. Economic circumstances were not favorable for its production, and further research was needed to obtain desired characteristics for human consumption.

Attempts are being made to use soybean flour in doughnut making. Federal regulations also permit its usage in sausage, 3.5% of the weight of the final product. Substantial quantities of soybean flour are now being added to pet foods. Total production was slightly over 60 thousand tons in 1953-54.

Dehulled extracted flakes are a source of various industrial proteins (wallpaper coating, paints, adhesives, whipping agents, foam stabilizers, etc.).

Soybean glue for soft plywood has averaged about 12 thousand metric tons annually in recent years. Smaller quantities are used for hard plywood. Both industrial proteins and glue represent only a minor outlet for the total soybean production. (See also discussions of use of flour and protein in Chapters 5, 10, 11, and 15.)

(3) *Soybean oil* (5). In its chemical properties soybean oil occupies an intermediate position between cottonseed oil and linseed oil; it has to compete with cottonseed oil in edible products and with linseed oil in products where drying oil is used, having more unsaturated fatty acids than the former and less than the latter. The fact, however, that soybean oil does not become yellow with age in paints, as linseed oil and tung oil tend to, has created great demand for it in the manufacturing of interior enamels. An enormous amount of research and improvements in refining techniques have made possible the use of soybean oil for edible oils and other industrial products.

Whereas the yield of oil per bushel of soybeans was only about 7.5 pounds in the early 1920's, it increased considerably by the late 1930's owing to the application of solvent-extraction equipment, and reached an average of 9.2 pounds in 1937-41. There was a decline in oil yield during the war years, but it began to increase in the postwar period, reaching a peak in 1948-49 of 9.8 pounds per bushel. This figure is certainly much higher (10.5 pounds per bushel) for individual plants using the solvent-extraction process.

In 1944-48 domestic consumption averaged nearly 1.4 billion pounds per year, which was larger than the production or disappearance of any other vegetable oil in the United States for the period, and it was equal to about 15% of total domestic disappearance of all fats and oils. The total 1954 production of 2.9 billion pounds was over 20% of all fats and oils produced, and the amount of soybeans exported in 1954 represented an oil equivalent of 550 million pounds of soybean oil.

Soybean oil has occupied first place in the shortening industry since 1935

and second in the margarine industry since 1936. Beginning in 1951, and especially in 1952-53, soybean oil has taken an increasingly larger share of the margarine market. In 1953 the consumption of bean oil in margarine amounted to about 720 million pounds, compared to about 275 million pounds of cottonseed oil (12). Soybean oil is widely used in other edible products such as cooking and salad oils, mayonnaise, and salad dressings. Fatty acids, sterols, and tocopherols are important by-products of soybean oil.

(4) *Soybean oil foots* (5). Foots, or soapstock, is the by-product that results from the alkali refining of soybean oil, averaging about 6% of the volume of crude oil refined. About 70 million pounds of soybean oil foots was used in recent years, mainly in soap manufacturing. Some of it is acidulated for fatty acid distillation to be used in special paints and varnishes, asphalt tile, roofing materials, and electric wire insulation.

(5) *Lecithin*. The product obtained by the so-called degumming of crude soybean oil is referred to in the trade as soybean lecithin. Lecithin is a complex mixture of phosphatides and other lipid and non-lipid materials. High-quality commercial lecithin contains 60 to 65% phosphatides and 35 to 40% soybean oil, depending on the consistency desired in the final product.

Soybean oil lecithin is widely used in the margarine, chocolate, confectionery, ice cream, macaroni, and baking industries. Its usefulness in pharmaceuticals, cosmetics, paints, rubber, and petroleum products, and in the leather and textile fields, is worth mentioning. It is also a good antioxidant.

The demand for lecithin has been merely a small fraction of the potential production of vegetable lecithin, indicating that considerable research is needed to find new applications for this product. Demand is better for lecithin products tailored for some specific purpose than for the crude lecithin, which needs considerable amount of processing before it can be used in a product. In 1948, 8 million pounds were produced (13), and production in 1954 amounted to 26 million pounds.

(6) *Other soybean products*. As a vegetable or other form of food, soybean does not have any importance in the United States. It plays a very important role in Asiatic foods, however, as is described in Chapter 9. Soybean plants as green manure, forage, and pasture, and soybean meal as fertilizer also have significance in Asiatic countries.

III. STRUCTURE AND COMPOSITION OF THE SOYBEAN SEED (14)

1. Gross and Microscopic Structure

Typical of a legume, the seed of the soybean has a smooth, often shiny, thick seed coat. The hilum or seed scar is elliptical in shape; the chalaza, a small groove at one end of the hilum, is the point where the

seed coat is connected to the body of the ovule. At the other end there is the micropyle which is a small orifice through which the primary root of the seedling makes its way in germination.

The seed coat or spermoderm has four layers of cells: (1) the palisade cells, 40 to 60 microns long, having a cuticular outer layer, colorless cell walls, and pigments in the cavity of colored varieties; (2) the hour-glass or column cells, which vary from 30 to 70 microns in size (very compressed on the ventral side), and have an I-shape which is characteristic of soybean structure; (3) the spongy parenchyma, which consists of six to eight layers of flat box-like cells of various size, mostly 40 to 60 microns; and (4) the aleurone layer, which is a single series of rather thick-walled cells filled with dense protein (aleurone).

At the area of the hilum, the above-mentioned structure is modified by the presence of a double layer of palisade cells, by the formation of sclerenchymatous cells with reticulate walls, and by the loose parenchyma of star-shaped cells.

Removal of the seed coat exposes the embryo which consists of the two thick seed leaves, cotyledons, which make up its bulk. The cotyledons are covered with cubical cells filled with grains of aleurone, and the inside is a conglomerate of layers of elongated palisade-like cells containing aleurone and oil. The remainder is the cylindrical hypocotyl rooted at the micropyle and having the leaf bud or plumule at the opposite end from the micropyle.

Soybean seeds of the most common commercial varieties weigh between 10 to 20 g. per 100 seeds. Seed size is dependent on environmental conditions and does not have any definite relationship to oil content. Shape and color vary considerably from strain to strain.

For details on the genetic characteristics of the soybean, *Glycine max* (L.) Merrill, the reader is referred to the work of Williams (15).

2. Influence of Variety, Soil, and Climate on Soybean Yield and Composition

Piper and Morse reported results of analyses made of over five hundred distinct kinds of soybeans by the U. S. Department of Agriculture, which show a range, on an air-dry basis, of 12 to 24% in the content of oil and 30 to 46% in the content of protein (16).

Table IV gives the range and average composition of ten common American varieties of soybeans and of the Illini variety grown at five locations in the United States during five successive crop years, as reported by Cartter and Hopper (17).

The same authors came to the conclusion that the oil content of soybean seed is a function of variety and that varieties and strains inherit their chemical composition. Although environmental factors pro-

TABLE IV
CHEMICAL COMPOSITION OF SOYBEANS, GROWN IN FIVE LOCATIONS IN FIVE CROP
YEARS^{a, b, c}

Component	Range of composition		
	Lowest (%)	Highest (%)	Average (%)
<i>Ten varieties</i>			
Ash	3.67	5.90	4.99
Crude oil	14.95	22.95	19.63
Crude fiber	4.34	7.60	5.52
Crude protein (N \times 6.25)	36.62	53.19	42.78
Sugars (total as sucrose)	2.70	11.97	7.97
Phosphorus	0.419	0.822	0.659
Potassium	1.29	2.17	1.67
Calcium	0.163	0.470	0.275
<i>Illini variety</i>			
Ash	4.17	5.49	4.81
Crude oil	18.30	21.76	19.99
Crude fiber	4.59	5.78	5.26
Crude protein (N \times 6.25)	38.25	44.95	42.59
Sugars (total as sucrose)	5.73	11.68	8.83
Phosphorus	0.456	0.744	0.623
Potassium	1.38	1.98	1.67
Calcium	0.181	0.356	0.252

^a According to J. W. Morse, in "Soybeans and Soybean Products" (K. S. Markley, ed.), Vol. 1, p. 141. Interscience, New York, 1950.

^b See also Table III, page 221.

^c Moisture-free basis.

duce some changes in this inherited composition, they do not influence the relative ranking of a variety in question when compared with other varieties in percentage of oil, protein, phosphorus, and calcium content. It was concluded that the performance of a variety or strain observed at one location suggests the relative performance of the same variety at another location. Cartter and Hopper's investigations also indicate that variety has more influence on chemical composition of soybeans than does environment.

Garner *et al.* (18) showed that all varieties responded to changes of environment in a like fashion as far as size and oil content of the seed were concerned; climate was more important for size and oil content than soil conditions.

Viljoen (19) worked out regression coefficients for oil and protein content and found that for every degree of rise or fall in minimum temperature a

corresponding increase or decrease of 0.44% in the oil content may be expected, and for every degree of fall in minimum temperatures an increase of 0.39% in protein content of the seed will follow. Webster and Kiltz (20), however, pointed out that in Oklahoma's warm climate soybeans have a lower oil and higher protein content than soybeans grown in other parts of the country.

It has been reported that phosphorus alone and phosphorus potassium combination as fertilizers improve the oil content of the beans more than a potassium-nitrogen combination; and a phosphorus-nitrogen combination improves the protein content more than phosphorus alone or in combination with potassium (19).

Lipman and Blair found that the addition of lime to the soil increased the percentage of nitrogen from 4.73 to 6.15 and the yields of seed from 13.2 to 19.3 bushels per acre (21).

Fellers found a direct relationship between lime addition and protein content and an indirect relationship between lime addition and oil content. Phosphate added to the limed soil produced an increase in the oil content, and the combined addition of potassium and nitrogen resulted in a slight decrease in oil content and increase in the protein content of the seed (22).

There are numerous findings similar to the above, but the complex nature of the problem makes it difficult to take all factors into consideration and at the same time evaluate the individual effects of a single factor. This field offers an unlimited opportunity and challenge for further research.

Besides the quantitative oil yield, the effect of variety and environment on the qualitative characteristics of soybean oil have been the subject of numerous investigations. Cartter and Hopper (17) observed that the iodine value of the oil is about equally influenced by variety and climate. They also concluded from data obtained on ten varieties grown at five locations for five consecutive years that the iodine value of the oil of a given variety is influenced by the prevailing temperatures during the period of seed development and oil metabolism, with high temperatures depressing and low temperatures raising the iodine value. Weiss *et al.* (23) found the following significant correlations: large seed size and low iodine number of oil; lateness of maturity and low protein content; low mean temperature during the period from flowering to maturity and high iodine number of oil; high oil content and low iodine number of oil; and high protein content with low oil content.

Similar differences can be found in the amino acid composition of soybean proteins. Csonka and Jones (24) report the following range of differences obtained on glycinin from ten varieties: 0.74 to 1.46% for cystine, 1.89 to 2.84% for tryptophan, and 2.29 to 3.10% for tyrosine.

For more detailed information the reader is referred to the works of Morse (16), Cartter and Hopper (17), and Weiss *et al.* (23).

IV. METHODS OF PROCESSING

By the mid-1930's the soybean processing industry in the United States was firmly on its feet, and markets for soybean oil and meal

were well developed. The first permanent solvent-extraction operation began in 1934. The sharp increase in soybean output in 1942 to 188 million bushels (from 107 million in 1941) created a difficult processing problem, since the quantity of soybeans available for crushing considerably exceeded processing capacity. Under government programs the surplus was sent to southern cottonseed mills. Although there was no possibility for expansion during the war, this situation was quickly remedied in the late 1940's. In 1948 the processing capacity in the United States was over 200 million bushels, and in 1951 it was around 310 million bushels per year. This compares with 243 million bushels actually processed in 1951 (25).

There are three major methods of processing soybeans: hydraulic press, screw press, and solvent extraction. These have been described in Chapter 4; the following discussion deals with specific operations involving soybeans. Hydraulic presses were used on a limited scale during World War II as an emergency measure and have no significance now in the United States. The continuous screw-press process was the chief means of processing beans prior to World War II, and then it was quickly replaced by solvent extraction. In the late 1940's about half of the total soybean crop in the United States was handled by the solvent-extraction process, and in 1951, 75% of the total crop was solvent-extracted (26).

Regardless of the type of oil extraction, the preparation of the soybeans is the same. The beans should preferably be cleaned, dried (if above 13% moisture), and blended by transfer from one bin to another to temper them to like conditions. They are transferred to the mill by belt conveyor and bucket elevator, collected in storage bins, weighed, and then fed through a surge hopper into the cracking rolls. The cracking is achieved by corrugated rolls, either two or three stands high, to provide a gradual size reduction. Many processors remove the hulls of soybeans since they are low in oil content and the capacity of the plant is increased by the percentage of the hulls removed. They are removed by air suction, then passed over screens to remove any meats, transported to a grinder, by-pass the extractor, and then fed back to the extracted meal.

It is necessary to reduce the moisture content of the beans to 2.5 to 3% for the screw press; for optimum operation of the solvent-extraction process, 9 to 10% moisture is desirable in the flakes. Preparatory to flaking, the cracked beans are conditioned to 160° to 170°F. in a rotary steam-tube dryer or a stacked kettle cooker, either of which may be equipped with water sprays so that moisture may be added if necessary. The cracked beans then are carried to the flaking machines having

smooth surface rolls which produce flakes of about 0.008 inch in thickness. The flakes are finally conveyed to the extractor (27).

In the hydraulic process the seed is pressed through standard five-high cottonseed crushing rolls, then through a conventional stack cooker, cake former, and hydraulic press with little deviation from the methods used for cottonseed. The press cakes are formed with about 5% moisture, and the yields are approximately 8.5 pounds of oil per bushel of soybeans (27).

In the screw-press process the moisture content of the cracked soybeans is reduced in a dryer to about 2.5 to 3%, and the beans are transported to the feeder of the press. Before the beans enter the barrel of the press, they are conditioned at 270 to 280°F. in order to achieve final drying and to prevent so-called "case hardening." In the barrel, the beans are immediately put under pressure, but at the beginning no shearing action takes place; shearing action starts after the beans have reached the successive screw section of the barrel, and the pressure increases steadily, forcing the oil out through the bars of the barrel. The high internal pressure and friction during the pressing operation generates heat. Overheating is prevented by circulating water in the barrel cage rings or by pumping the oil from the screening tank through a water-cooled heat exchanger and reverting it through specially designed spreaders over the barrel. The cake thus obtained contains about 4% of oil (27).

After many decades of experience and unsuccessful attempts, the first large-scale continuous solvent-extraction plants in the United States were introduced from Germany in 1934. Both the Hildebrandt "U"-type and the Hansa-Mühle or Bollman basket-type extractor were used exclusively until 1937 when the vertical gravity extraction columns built by the Allis-Chalmers Manufacturing Co. and the V. D. Anderson Co. were made available. Modifications of the Hansa-Mühle or Bollman extractor have been built in the United States by the French Oil Mill Machinery Co. and the Blaw-Knox Co. (28). Modifications and improvements in extraction units have been made to increase efficiency, dependability, simplicity, and safety.

Considerable research was devoted to find the most efficient and practical solvent for the extraction. Because of its nonflammability, trichloroethylene appeared to be promising at one time. After toxic symptoms were observed in cattle fed trichloroethylene-extracted meal, however, it lost popularity in the soybean-processing industry (29). (See also Chapter 6.) Hot alcohol extraction was tried in Manchuria, but it is impractical in the United States because of its high cost, the necessity of drying the flakes to 3% moisture in the recovery process, the difficulty in maintaining high purity, and the poor selectivity of alcohol as solvent.

Prior to 1930 no petroleum company specialized in solvent-extraction naphthas. Increasing demand was followed by the development of

naphthas of narrow boiling range with negligible evaporation residue, odor, or taste, relatively free from sulfur compounds and unsaturated compounds which tend to form gums. Finally, commercial hexane filled the requirements better than any other solvent tried, because of its low cost, easy recovery, and selectivity for vegetable oils.

Extreme precautions are necessary with flammable hydrocarbon solvents. Proper installation and intelligent operation reduces this danger to the minimum. Various designs have been devised for ventilation, fire control, flame arresters, and sewer traps to prevent accidental spillage escaping to the sewers, and for safety tools. Electrical equipment should be explosion-proof, designated as Class 1, Group D, of the Underwriters' Laboratories, whenever contact with flammable gases is possible.

In the United States the bulk of soybeans processed by solvent extraction is handled by the basket and vertical gravity types. Most of the plants operate at or near a solvent-to-soybean ratio of 1:1.

In the Bollman system the flakes move in sieve-bottomed baskets attached to an endless chain similar to a bucket elevator. The charge of flakes is dumped into the basket from the filling device which also acts as a vapor lock. The solvent drains in both concurrent and countercurrent directions (30). When the flakes enter the extractor, they are immediately sprayed with half-miscella. Solvent is added at the top of the ascending side of the cycle. Both half-miscella and solvent drips through the baskets below to the floor pan, resulting on the descending side in full-miscella (up to 28% oil) and on the ascending side in half-miscella (up to 15% oil). The half-miscella is pumped to a storage tank from where it can be used for spraying the descending baskets. The full-miscella is pumped through filters to a small surge tank and then to evaporators where the separation of oil from the solvent begins. The final step in solvent recovery from the oil occurs in a vacuum stripping column, and the finished oil is pumped from the last section of the stripping column to storage tanks. At the time the basket carrying the extracted flakes reaches the top of the cycle, it is automatically inverted. The flakes saturated with solvent are dumped in a bin and carried away by paddle conveyors to solvent recovery dryers or desolventizers. Blaw-Knox Co. has developed a horizontal basket or all-type extractor, known as the Rotocel. Another horizontal design was also offered by the French Oil Mill Machine Co.

In the Hildebrandt system, the solids are moved by screw conveyors in a U-tube while the solvent is circulated in a countercurrent direction (28). The vertical gravity-type extractor uses a single column containing slotted adjustable plates. The flakes drop downward from one plate to another after being carried around the circumference of each plate by a scraper arm. Solvent is introduced at the bottom and flows countercurrently, and the miscella flows out at the top. At the time the flakes reach the bottom they are free of oil and enter the discharge screw which forces the plug of flakes against a pressure-loaded cone. The solvent is increasingly enriched with oil as it flows upward into a sealing chamber, where the separation of fines takes place. The miscella is then filtered and distilled.

In modern plants the foregoing operations and their coordination are fully automatic.

After the wet flakes are dumped they are carried to the desolventizing or drying operation. There are three general types: (1) horizontal, steam jacketed, (2) recycled vapor, and (3) vertical, direct steam.

Prior to 1951 the most popular equipment was the horizontal-type, steam-jacketed paddle conveyors (Schneckens), usually arranged one above the other in banks of several units. The meal passes from these units into a deodorizing drum, where the last traces of solvent are removed from the flakes before they are discharged. There are various devices to take off the vapor efficiently. Direct steam is usually introduced near the bottom outlet of the bank of dryers to displace the solvent vapor in the lower tubes and allow complete effusion of the solvent from flakes. Water may be added to the meal in the lower tubes to agglomerate fines, thus eliminating the dusty condition to some extent. Sometimes a very slight vacuum is drawn on the bank of dryers, but in most instances atmospheric pressure is used.

In the recycled solvent vapor system for the desolventizing of solvent-extracted particles, Leslie (31) devised a method whereby solvent-extracted soybean flakes are desolventized continuously by a rotating-drum desolventizer in which a part of the effluent vapors from the drum are superheated by an external heater and recirculated through the drum. The final solvent is stripped from the meal by live steam added either in a special section of the desolventizer or in a separate "stripper."

The vertical-type desolventizer is a multiple-section vessel containing several pan sections in vertical arrangements. Direct steam is used to drive off the solvent. (See also Chapter 10 for a discussion of desolventizers.)

Whereas screw-pressed meals are exposed to high temperature (300°F.) for short periods during processing, solvent-extracted meals have to go through the so-called toasting process in order to attain the optimum nutritive value. There are several types of toasters, including vertical-stacked cookers, rotary tubular cookers, and pressure cookers.

A method described by Kruse (32) is gaining acceptance because it combines desolventizing and toasting in a single vessel and it produces a meal of superior quality. This method employs a steam-jacketed cooker which consists of several compartments having openings between the compartment floors. A rotating driven member spreads and discharges the flakes through the openings. The flakes are passed downward through the compartments and finally leave the unit at the bottom of the last compartment.

The sequence of steps in this process is as follows: Flakes, as they come from the extractor, contain 30 to 37% solvent. As they are rapidly heated with direct steam and agitated in the upper compartments, the moisture content of the flakes is increased to 17 to 20% owing to steam condensation. Jacket heat is then applied, and toasting and drying proceed in the lower kettles of the unit where the temperature is increased to 220° to 230°F. The moisture content is reduced to 13 to 16% before the meal is discharged. This type of toasting is applicable where solvent is removed prior to the addition of moisture to the flakes or where solvent-saturated flakes are introduced directly to the cooker and moisture is added within the cooker. The nutritive value of the meal in this process is greatly enhanced by the efficient destruction of anti-trypsin activity, yet essential nutrients including high thiamine levels remain well preserved.

The Kruse process is further described by Hutchins (33), who disclosed specific equipment design for carrying out the process. The successive series of closed chambers of the toaster are connected by valve-controlled passages. Filtration of the solvent vapors prior to withdrawal, reducing the amount of dust going to the condenser, and regeneration of the moisture as steam for recondensation are additional features of this equipment.

The toasted meal is then dried, cooled, and ground in hammermills to a size which makes it easy to handle and acceptable to the trade. It is then separated on screens, the coarse particles are reground, the dust is further processed, and the meal of suitable size is now ready to be stored as finished meal.

Several attempts have been made to correlate toasting efficiency and nutritive value by *in vitro* laboratory tests (34, 35). (See also Section VI of this chapter.) The most widely used methods measure urease activity of the meal, amount of trypsin inhibitor destroyed in the toasting process, water- and alkali-soluble fractions of the meal protein, fluorescing properties of the particles, amino acid content of the meal with particular reference to the sulfur-containing and dibasic amino groups, and finally availability of these amino groups to proteolytic enzymes or microorganisms. All these methods have been evaluated by the authors of this chapter.* Good correlation was found within a given process between processing conditions and laboratory tests; not very satisfactory correlation was found between *in vitro* tests and nutritive value; and no correlation existed between laboratory results and nutritive value when samples were supplied by different processors.

As stated previously, practically all the bean processing presently carried on in the United States is by the extraction method. The shift from the screw-press process to extraction, although accelerated by improved extraction technology and the remarkable adaptation of the soybean material to a mechanized extraction process, resulted mainly from

* W. W. Cravens, and E. Sipos, Central Soya Co., Inc. (Unpublished Data), 1955.

economic factors. The extraction process yields 20 to 30% more oil, and the cost to process a bushel of beans by this method is only slightly above screw-press processing costs. Thus with oil priced at three to five times the price of meal, the increased margin resulting from the extraction operation put the screw-press process in a less favorable position.

The soybean industry in the United States is notably different from other oilseed industries in the number of plants processing at very high capacities. It is estimated that at least half of the beans processed in this country are processed in plants having installed capacities each in excess of 300 tons of beans per day, and there are two plants each of which processes over 1000 tons of beans per day. For more information concerning the trends in the oilseed industry the reader is referred to the work of Goss (36).

V. COMPOSITION OF SOYBEAN OIL MEAL

1. Standard Specifications in the United States

The following definition and standard specifications are quoted from the trading rules of the National Soybean Processors Association: "Soybean Oil Meal is the ground residue after the removal of the oil by pressure or extraction from soybeans. The name descriptive of the process of manufacture, such as expeller,* hydraulic, or solvent extracted shall be used as part of the brand name. It shall be designated and sold according to its protein content."

		Soybean oil meal		
		41%	44%	50%
Protein, %	Minimum	41.0	44.0	50.0
Fat, %	Minimum	3.5	0.5	0.5
Fiber, %	Maximum	7.0	7.0	3.0
Nitrogen-free extract, %	Minimum	27.0	27.0	27.0
Moisture, %	Maximum	12.0	12.0	12.0
(When shipped by seller)				

2. Soybean Protein

Proteins of soybeans change their native characteristics in the processing of the beans. Hence proteins of the meal are not identical with the isolated fractions of the seed.

* "Expeller" is the name used and copyrighted by V. D. Anderson and Co., Cleveland, to describe the screw press which they manufacture. In much of the literature and even in some trading rules, this word is used synonymously with the term screw press.

Muramatsu analyzed a sample of dry soybeans which contained 6.94% nitrogen. Of this, 5.97% was soluble in water, 0.26% in saline solution, 0.16% in alkali, and 0.55% remained in the residue. The water-soluble portion contained 84 parts globulin, 5.36 parts albumin, 4.36 parts proteose, and 6.03 parts non-protein nitrogen (37). Tadokoro and Yoshimura called the water-soluble and dialysis-precipitated protein fraction glycinin A. Glycinin B was obtained by extracting the residual meal, after extraction of glycinin A, with 10% sodium chloride solution; and glutelin by using 0.2% sodium hydroxide to extract the residual meal after removal of glycinin B. Legumelin was derived from the filtrate from glycinin A by precipitation with ammonium sulfate (37). Jones and Csonka separated five fractions from a 10% sodium chloride extract of soybean meal by precipitating with successively higher concentrations of ammonium sulfate. The fraction separated at 55% saturation mostly resembled the globulin-like protein found and named glycinin by Osborne and Campbell in 1898 (38). None of these fractions has actual chemical individuality, since they may vary between varieties quantitatively, and in amino acid content. A survey of soybean protein fractions and their electrophoretic patterns was made by Smith *et al.* (39).

Although the generally accepted nitrogen factor for converting the Kjeldahl nitrogen values of soybeans or soybean oil meal to protein is 6.25, many suggestions have been made to change this factor to conform to the actual nitrogen content found in various, presumably pure, soybean proteins. Owing to the presence of non-protein impurities and the different ways of fractionation of soybean proteins there is some uncertainty about the actual nitrogen content of soybean proteins. Csonka and Jones (24) reported 17.74%, whereas Smiley and Smith (40) obtained only 16.90% from ethanol-extracted Willomi soybeans. Jones (40*a*) recommended a conversion factor of 5.71. In view of these differences, however, Smiley and Smith (40) considered the adoption of a new factor to be of little value.

Extraction of the oil and subsequent toasting alters the native protein of soybeans in many ways so that any classification has to be made according to the method of separation. According to Nakajima, the water-soluble glycinin is denatured almost completely into a glutelin-like protein (soluble in 0.2% sodium hydroxide) during extraction of the oil (41). Evans and St. John, studying the effect of heat treatment on soybean meals, also found the drastic decrease of the water-soluble nitrogen fraction of the meal and its conversion to an alkali-soluble fraction. Even this alkali-soluble fraction is further insolubilized on excessive heating (42). Although heat causes the most important

changes in protein structure, storage, exposure to solvents, and grinding have similar but much milder effects. (See also Chapter 5.)

3. Amino Acids

Properly processed soybean oil meal is one of the best sources of essential amino acids for animal feeds. Soybean oil meal is not the best source, however, of sulfur-containing amino acids. The sulfur-to-nitrogen (S-N) ratio in soybean oil meal is approximately 1:17, as

TABLE V
AMINO ACID COMPOSITION OF SOYBEAN OIL MEAL
(Grams per 16 grams of nitrogen)

Amino acids	Reference		
	a	b	c
Arginine	7.0	6.0	7.5
Histidine	2.5	2.3	2.5
Lysine	6.6	6.4	6.2
Tyrosine	3.2	3.1	—
Tryptophan	1.2	1.2	1.7
Phenylalanine	4.8	4.8	4.9
Cystine	1.2	—	—
Methionine	1.1	0.7	1.4
Threonine	3.9	3.8	4.0
Serine	5.6	—	—
Leucine	7.6	6.6	7.7
Isoleucine	5.8	6.4	5.5
Valine	5.2	5.0	5.4
Glutamic acid	18.5	—	—
Glycine	8.3	—	—
Alanine	3.8	—	—
Proline	5.4	—	—

^a R. J. Block and K. W. Weiss, "Amino Acid Handbook," Charles C Thomas Publishing Co., Springfield, Ill., 1956.
^b H. H. Williams, *Cornell Univ. Agr. Expt. Sta. Memoir* 337 (1955).
^c C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008, 1956.

compared to 1:7 in alfalfa meal, 1:6 in corn gluten feed, 1:15 in cottonseed meal, and 1:14 in linseed meal (43).

The amino acid composition of soybean oil meals is given in Table V. In addition to the absolute content of amino acids, importance is attached to the concept of availability of amino acids since the discovery of the beneficial effect of heat treatment on the nutritive value of soybean oil meal. This has led to an interest in elucidating the mechanisms involved during the enzymatic digestion process *in vivo*.

Ichikawa and Yoshinaga (44) demonstrated that acid hydrolysis of soybean proteins liberates acidic amino acids first; then neutral, hydroxy, and basic amino acids are released, in that order. Amino acids with aromatic, heterocyclic, and sulfur-containing groups are liberated more slowly than other amino acids. Peptides containing tyrosine, histidine, proline, and valine are hydrolyzed with difficulty.

Riesen *et al.* (45) determined the proteolytic action of pancreatin on raw, properly heated, and overheated soybean oil meals and found that the proteolytic activity of pancreatin was considerably lower in mixtures containing the raw meal than in meals which have been autoclaved, apparently owing to the effect of the trypsin inhibitor in the meal. The results of Riesen *et al.* also demonstrated that meals subjected to dry heat or excessive toasting show a decrease in the availability of amino acids to pancreatic digestion; the dibasic amino acids including the nutritionally important lysine suffer especially. Similar findings have been reported by Block *et al.* (46) and by Evans *et al.* (47, 48).

4. Suppressive, Toxic, and Other Factors

Among the nitrogenous constituents in the meal there are inhibitory, toxic, and stimulatory factors. An antioxygenic factor was found to prevent fats and oils from deterioration (49). Rubin describes an allergenic factor causing asthma by soybean dust (50). Soybean meal extract was noted to promote plant growth and fermentation (51). Wilgus *et al.* attributed a goitrogenic substance to soybean meal (52). O'Dell *et al.* reported moderate amounts of an antithyrototoxic activity in soybean oil meal (53). Souza (51) described a blood coagulant, and Crexatto (51) demonstrated an anticoagulant action in soybean. The latter was identified with the trypsin inhibitor.

Trypsin inhibitor is considered the most important of this group, since its destruction by heat treatment greatly increases the nutritive value of soybean oil meal. Counteracting trypsin in the intestinal tract slows down the *in vivo* release of amino acids. The increased efficiency of pancreatic hydrolysis on the liberation of essential amino acids in cooked meals in contrast to the raw meal was mentioned in the foregoing (45).

Viswanatha *et al.* described the proteolytic inhibitors in soybeans strictly as antiproteases having no effect on peptidase activity (54). Kihara *et al.* reported the purification of a peptide factor for *L. casei* from soybean protein (55), and Indian workers found the streptogenin activity in soybeans for *L. casei* several times higher than in casein (56).

5. Enzymes

Urease is important among the enzymes in soybean meal because of its effect on feeds containing urea. Other enzymes include allon-toinase, amylase, ascorbic acid oxidase, carboxylase, catalase, β -glycosidase, glyoxylase, lipase, and lipoxidase. Soybean meal contains also a mixture of different proteolytic enzymes, such as cathepsin, a polypeptidase, and a dipeptidase (51).

Non-protein nitrogenous substances are present in small amounts and include free amino acids, amides, polypeptides, peptones, organic nitrogen bases, and other organic and inorganic nitrogen compounds.

6. Carbohydrates

Reports about the carbohydrate content of soybean meal are controversial. About half the crude fiber occurs in the hulls as cellulose or other polysaccharides, and the rest is in the cell walls of the cotyledons, along with galactans, pentosans, and unidentified hemicelluloses (57). According to Tscherniak the crude fiber of the soybean consists of 82% cellulose, 11% pentosans, 6% lignin, and 1% protein (58). Most of the sugars present in soybean meal are polysaccharides including di-, tri-, and tetrasaccharides. Stachyose, raffinose, and sucrose are reported in small amounts. The presence or absence of small quantities of starch depends on variety. Burrell and Wolfe report 4.1% reducing sugars and 5.2% sucrose (moisture-free basis) in soybeans (59).

Kawamara found 20% reducing sugars after hydrolysis in defatted soybeans of 12% moisture and 30% nitrogen-free extract (60). Soybean oil meal having a 50% protein and a 30% carbohydrate content was found to contain 6.8% sucrose, 4.1% galactan, 4.7% araban, and 4.6% crude fiber (61).

Adolph and Kao claim that soybean carbohydrates are in between cellulose and starch in their digestibility by rats; about 40% of the total can be utilized by the animal (62). Soybean meal also contains various types of glycosides such as phytosterolins, saponins, genistein, and isoflavone glycoside.

7. Vitamins

Soybean oil meal is not a rich source of vitamins, but because of its extensive use in feeds, it contributes significantly to meeting the vitamin needs of poultry and swine. Soybeans would be a good supply of thiamine; however, this is the vitamin most likely to be destroyed by the heat treatment of the meal. Cooking at high moisture level with live steam preserves more thiamine than any other process; values usually

run over 7 γ /g. on such meals. Available information on the vitamin content of soybeans and soybean oil meal is given in Table VI. Soybeans also contain 1.9 γ /g. of vitamin K (62a); soybean oil contains 1.4 γ /g. of vitamin E (62b); the linoleic acid content of the oil is 50 to 60%; and the linolenic acid content is 2 to 8% (62c).

8. Minerals

Soybean oil meal is a fair source of phosphorus, but, as with other products of vegetable origin, much of this phosphorus is in phytin or organic form and thus relatively unavailable to the animal. The hydrolytic breakdown products of soybean phosphatides consist of inositol monophosphate and a mixture of phosphoric acid esters (63). Soybean oil meal, like most oilseeds, is low in calcium content. The mineral content of soybeans and soybean oil meal is given in Table VII.

VI. SOYBEAN OIL MEAL FOR FEED

1. General Considerations

The use of soybean oil meal for feed, as with all feed ingredients, depends on (1) nutritional entities present, (2) availability of nutritional factors to the animal, (3) palatability or acceptability of ingredient to the animal, and (4) the presence or absence of any toxic materials in the ingredient. These criteria must be evaluated for each species, and thus sweeping generalizations about the nutritional value of any ingredient are never justified. Soybean oil meal has been included extensively in feed formulations for many years; innumerable investigations of its nutritive value in various types of formulations have brought to light many virtues and shortcomings and have thus provided a more scientific basis for its use in rations for livestock and poultry.

A review of the literature reveals that many of the milestones of nutrition were reached in the course of experiments wherein soybean oil meal replaced varying quantities of ingredients of animal origin in livestock and poultry rations. Studies on the need for and requirements of minerals and vitamins by animals and poultry were intensified through difficulties that arose when soybean oil meal replaced ingredients of animal origin. For many years it was thought that inferior results were obtained when soybean oil meal served as a source of much of the supplemental protein because of its inferior protein quality. Perhaps this was true in many of the earlier experiments, owing to a lack of knowledge regarding optimum processing conditions. It became increasingly evident, however, that vitamin and mineral factors were involved as well as protein quality in rations containing primarily soybean oil meal as the source of protein. Many experiments on methods of processing soybeans to bring out the inherent quality of the protein have been reviewed by Hayward (64).

TABLE VI
VITAMIN CONTENT OF SOYBEANS AND SOYBEAN OIL MEAL

Vitamin	Soybeans			Soybean oil meal					
	γ/g.		γ/g.	41% ^a		44% ^a	45-50% ^a		50% ^a
	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
Thiamine	17.5	12.3	11.0 ^b	4.1	—	—	7.0-12.0 ^f	—	—
Riboflavin	3.6	3.4	—	3.1	2.7	2.2	—	3.3	3.3
Pyridoxine	11.8	—	7.1-12.0 ⁱ	—	4.6	4.9	3.1-5.3 ^f	5.1	5.1
Biotin	0.80	—	—	—	—	—	—	—	—
Pantothenic acid	21.5	13.0	15.7 ^j	15.0	12.6	17.2, 14.0-16.0 ^e	13.3 ^g	16.1	16.1
Folic acid	1.9	—	2.1 ^k	—	4.9	4.0	—	4.2	4.2
Niacin	21.4	23.0	23.0 ^l	40.0	19.0	21.4	15.5-28.7 ^f	23.4	23.4
Inositol	mg./g. 2.3	—	mg./g.	mg./g.	mg./g.	mg./g.	—	mg./g.	mg./g.
Choline	—	—	3.4 ^m	—	2.1	1.9	—	1.8	1.8
Carotene	units/g. 3.5-11.5	units/g. 1.2	—	—	2.9	3.3	—	4.1	4.1
References	^b	^c		^c	^d	^d	—	^d	^d

^a Refers to percentage of protein (N × 6.25) in meal.
^b P. R. Burkholder and I. McVeigh, *Plant Physiol.* **20**, 301 (1945).
^c National Research Council, "Composition of Feeds, Suppl. to Report No. 1, Vitamin and Mineral Contents." Washington, D.C., 1947.
^d A. E. Staley Mfg. Co., *Research Bull. No. 203*. Decatur, Illinois, 1954.
^e G. M. Briggs and F. S. Daft, in "The Vitamins" (W. H. Sebrell, Jr., and R. S. Harris, eds.), Vol. 2, p. 642. Academic Press, New York, 1954.
^f Central Soya Co., Inc., Decatur, Ill., Vitamin Laboratory (thirty-three commercial samples from different commercial sources).
^g Central Soya Co., Inc., Decatur, Ill., Vitamin Laboratory (average of several production samples).
^h J. C. Fritz, *Trans. Am. Assoc. Cereal Chemists* **12**, 60 (1954).
ⁱ E. E. Snell and C. S. Keevil, Jr., in "The Vitamins" (W. H. Sebrell, Jr., and R. S. Harris, eds.), Vol. 3, p. 257. Academic Press, New York, 1954.
^j G. M. Briggs and F. S. Daft, in "The Vitamins" (W. H. Sebrell, Jr., and R. S. Harris, eds.), Vol. 2, p. 642. Academic Press, New York, 1954.
^k E. L. R. Stokstad, in "The Vitamins" (W. H. Sebrell, Jr., and R. S. Harris, eds.), Vol. 3, p. 170. Academic Press, New York, 1954.
^l J. M. Hundley, in "The Vitamins" (W. H. Sebrell, Jr., and R. S. Harris, eds.), Vol. 2, p. 542. Academic Press, New York, 1954.
^m W. H. Griffith and J. F. Nye, in "The Vitamins" (W. H. Sebrell, Jr., and R. S. Harris, eds.), Vol. 2, p. 60. Academic Press, New York, 1954.

TABLE VII
MINERAL CONTENT OF SOYBEANS AND SOYBEAN OIL MEAL

Mineral	Soybean	Soybean oil meal			
		Screw-pressed	41% ^a	44% ^a	50% ^a
		%	%	%	%
Calcium	0.22	0.26	0.28-0.31	0.30-0.33 ^b	0.37
Phosphorus	0.59	0.62	0.60-0.63	0.62-0.65	0.75
Sodium	0.38	0.14	—	—	—
Chlorine	0.02	0.01	—	—	—
Potassium	2.09	2.06	—	—	—
Magnesium	0.24	0.30	—	—	1.81
Iron	0.008	0.014	—	—	0.26
Silicon	—	0.1-0.3	—	—	—
Silica	—	0.03	—	—	—
Sulfur	—	—	—	—	—
Copper	12	p.p.m.	—	—	—
Manganese	32	14	—	—	—
Cobalt	—	29	—	—	—
Molybdenum	—	0.11	—	—	—
Boron	—	1.0	—	—	—
Nickel	—	14-18	—	—	—
Zinc	—	4	—	—	—
Iodine	—	18-52	—	—	—
Aluminum	—	—	—	—	—
References	c	e	e	e	f

^a Refers to percentage of protein (N × 6.25) in meal.

^b Solvent-extracted.

^c H. H. Mitchell, in "Soybeans and Soybean Products" (K. S. Markley, ed.), Vol. 1, p. 412. Interscience, New York, 1950.

^d W. J. Morse, in "Soybeans and Soybean Products" (K. S. Markley, ed.), Vol. 1, p. 148. Interscience, New York, 1950.

^e J. W. Hayward, in "Soybeans and Soybean Products" (K. S. Markley, ed.), Vol. 2, p. 899. Interscience, New York, 1950.

^f A. E. Staley Mfg. Co., *Research Bull. No. 203*. Decatur, Illinois, 1954.

Osborne and Mendel (65) showed in 1917 that heating soybeans improved their nutritive quality. This finding was confirmed by numerous investigators, and it was subsequently shown that adding cystine to diets containing raw soybeans markedly improved animal growth (66). Hayward and Hafner (67) reviewed the earlier experiments on the effect of heat on protein quality of soybeans and reported that methionine additions to practical rations containing raw soybeans markedly improved chick and rat growth. An improvement in growth was also observed when the sulfur-containing amino acids were added to a diet containing cooked soybeans. It was suggested that the entire protein fraction of the soybean was improved by heating. The protein quality of soybean oil meal was further studied by Almquist *et al.* (68), who concluded that properly heated soybean protein is slightly deficient in methionine but is complete in respect to all other amino acids required by the chick. In these experiments soybean oil meal served as the sole source of protein in the chick diet in contrast to most previous experiments where it served only as supplementary protein to other ingredients. Hence these were critical experiments on the quality of soybean oil meal protein for growing chickens.

The first evidence helping to explain the low biological value of raw soybeans was offered by Ham and Sandstedt (69), who reported the presence of a trypsin-inhibiting substance in raw soybeans and found that this substance had a retarding effect on the growth of chicks when included with a ration of autoclaved soybean oil meal. The experiments of Riesen *et al.* (45) definitely proved that the liberation of essential amino acids by pancreatic hydrolysis is increased by the heat destruction of the trypsin inhibitor. Liener found that the inhibitory effect in feeding tests is attributable only about half to the trypsin inhibitor, the other half being the effect of presence of another toxic substance, soyin (70). Blood-clotting time of chicks was observed to increase by feeding diets containing unheated, solvent-extracted soybean meal, and addition of vitamin K did not overcome this condition. The anticoagulant factor, however, was completely destroyed by autoclaving (71). A mixture of essential amino acids added to the diet failed to eliminate the retardation of chick growth caused by unheated soybeans, indicating that growth retardation is not entirely due to proteolytic inhibition (72). There is no doubt that soybean oil meal may contain toxic and growth stimulatory factors unknown at present. Their presence would increase or decrease the nutritive value of the meal under various conditions.

Proper toasting conditions (time, temperature, and moisture control) are of paramount importance in producing soybean oil meal of maximum nutritional quality. The experiments of Wilgus *et al.* (73), Hayward and coworkers (74), and numerous other investigators clearly demonstrate the relationship between heat treatment and nutritive value. (See also Table I, page 85.) That moisture level in the toasting step is highly important was demonstrated by Kruse (75), who developed a procedure for adding moisture to extracted soybean flakes prior to toasting. (See Section IV.) The large concentration of moisture present during this process facilitates destruction of the trypsin inhibitor in less time and with less heat, and before other vitamins and

essential amino acids are destroyed or rendered unavailable to the animal. Clandinin *et al.* (76) also reported that the deleterious effect of overheating extracted soybean flakes was greatly reduced by high moisture levels. Liener and Fevold pointed out that, in addition to the inactivation of the inhibitory substances, alterations in soybean protein per se as a result of heat may change its susceptibility to enzymatic breakdown (77).

It is well known that heat in the presence of moisture denatures proteins. That protein is altered is shown by its reduced dispersibility, by its solubility in water (78) (see also Table II and Fig. 1, Chapter 10), by the changes occurring to sulfur-containing functional groups (67), by the changes of its isoelectric point, by its hydrolytic breakdown (79), and by its interaction with carbohydrates (80). In overheated meals, there is some disappearance of amino groups indicated mainly by the destruction of lysine (81) and arginine. Newer methods of quantitative assessment of aminoid and carboxoid residues show that no methionine residues are directly exposed as the result of toasting. N-terminal lysine was found in both raw and toasted materials; this terminality suggests that susceptibility of lysine to destruction during overheating is due to the fact that a large part is in a relatively exposed position in the protein molecule (82).

McGinnis and Evans (83) could not obtain any improvement in growth by the addition of lysine, methionine, or cystine or by a combination of these amino acids added to a diet containing soybean meal autoclaved at 100° for 30 minutes. Autoclaving the meal at 130° for 60 minutes decreased the nutritive value, and this impairment was not corrected either by the single addition or by the combination of lysine, methionine, and cystine. It was concluded that prolonged autoclaving affects the availability of other nutrients besides the three amino acids mentioned. Evidence indicates that the sulfur-containing amino acids cystine and methionine form linkages resistant to enzymatic hydrolysis (84, 85). Several authors (86, 87) have pointed out the deleterious effect of interaction between amino acids and carbohydrates. Evans and Butts found that 40% of the lysine of soybean oil meal was destroyed by autoclaving for 4 hours, but soybean protein alone, when autoclaved, showed little loss of lysine. Similar observations were made for arginine, tryptophan, and histidine (88). The destruction of certain vitamins would also decrease the value of soybean meal. This is especially true for thiamine, which is considerably destroyed by improper heat treatment (89). The exact mechanisms of upgrading protein quality of soybeans by proper heat treatment remains somewhat obscure but has been the subject of extensive investigation. The reader is referred for more

information on this subject to the review published by Griswold (90) and to the general discussion of the heat effect in Chapter 5.

Several reports appearing between 1937 and 1944 indicated that fish meal was the best practical feedstuff to use in combination with all-vegetable diets in order to obtain best chick growth (91-93). In these experiments riboflavin was added to the experimental diets in pure form and at adequate levels, thus eliminating a possible deficiency of this vitamin. In many prior experiments the substitution of soybean oil meal for products of animal origin in practical rations had resulted in inadequate riboflavin levels and consequently poor results. That riboflavin was an important consideration in replacing animal products with soybean oil meal was established by experiments at Cornell (94). These experiments also provided information on the riboflavin content of practical feed ingredients, thus enabling subsequent investigators to eliminate the possibility of a riboflavin deficiency in experiments on soybean oil meal. Unfortunately it was assumed that the beneficial effect of fish meal, as well as other animal products, was due largely to the supplementary value of the protein supplied by the animal products. It became increasingly evident during the period 1940-1945, however, that rations for poultry and swine composed of all-vegetable ingredients, containing soybean oil meal as the major high-protein supplement, were deficient in one or more unidentified factors (95-98). Continued experiments on all-vegetable diets and the development of a microbiological assay for the unidentified factor led to the isolation of vitamin B₁₂ in 1948 (99). The rapid development of adequate and standardized supplies of this vitamin has given great impetus to the use of soybean oil meal in poultry and swine rations. Soybean oil meal is deficient in this critical vitamin, and adequate supplies made possible a more complete fortification of poultry and swine rations containing soybean oil meal as the chief high-protein supplement.

In subsequent experiments the addition of certain vitamin B₁₂ supplements to all-vegetable rations for poultry and swine was found to result in a greater growth stimulation than could be accounted for on the basis of their vitamin B₁₂ content (100). This led to the hypothesis that there were other unidentified factors necessary on all-vegetable diets. Further work revealed that antibiotic was present in certain vitamin B₁₂ supplements and that the pure antibiotic stimulated growth in chicks (101). Further studies have shown that growth of other species is stimulated by antibiotic feeding.

There remains, however, considerable evidence that there are still unidentified factors necessary for chicks fed all-vegetable rations. Sources of these factors are reported to be fish meal, condensed fish

solubles, liver, distillers' solubles, dried whey, brewers' yeast, forage juice, and certain fermentation residues (102). Recently the ash of distillers' dried solubles has been reported to stimulate growth in chicks (103, 104). It was reported that the ash of distillers' solubles gave a growth response about one-half that of the unashed material. The significance of the reports on unidentified factors remains to be determined, but it appears that there are as yet inorganic and organic unidentified factors.

It seems safe to state that the further isolation and identification of nutritional factors will be of great value in making possible the more efficient utilization of soybean oil meal in formulating livestock and poultry feeds. At the present time, however, we must rely on a variety of feed ingredients to supply the unidentified factors required by poultry and swine.

2. Soybean Oil Meal for Poultry

The isolation and identification of vitamin B₁₂ and the ready availability of other crystalline B vitamins has provided a new basis on which to formulate poultry rations with soybean oil meal. Much of the older literature on soybean oil meal in poultry feeding has been reviewed by Ewing (105), Heuser (106), and Hayward (64). The present trend is to use soybean oil meal to provide supplementary protein to cereal grains and grain by-products and to add known amounts of vitamins, minerals, and certain feed ingredients as sources of unidentified factors.

The principle limiting factors in simplified corn-soybean oil meal rations for poultry are vitamin B₁₂ (107), riboflavin (94), niacin, pantothenic acid (108), and certain unidentified factors (103). As previously stated, the chief limiting amino acid of properly processed soybean oil meal is methionine. Recent work (109) indicates that the need for methionine may vary with the productive energy level of the diet; as the level of productive energy of the diet is increased, the protein remaining constant, there is a commensurate increase in the need for methionine. This same principle will probably apply to other amino acids, but the effect on methionine is most obvious because of its presence in borderline amounts in practical chick starting rations containing soybean meal. (See Chapter 13.)

Natural feedstuffs that are good sources of methionine are sesame meal, fish meal, and corn gluten meal (110). All have been demonstrated to supplement soybean oil meal effectively in poultry feeding (91-93, 111, 112). Recent experiments (113, 114) also indicate that certain combinations of soybean oil meal and degossypolized cottonseed

meal provide a good amino acid balance for starting and growing chicks. Cottonseed meal is not so high in lysine and contains slightly more methionine than soybean oil meal. Thus these two protein feedstuffs may effectively supplement each other.

Soybeans and soybean oil meal are good sources of essential minerals as compared to grains and most of their by-products. When soybean oil meal replaces feedstuffs of animal origin, however, calcium and phosphorus adjustments must be made in the ration. The importance of adding minerals to a corn-soybean ration for poultry was demonstrated in 1922 (115). Numerous investigations on mineral supplements for corn-soybean oil meal rations have been summarized by Heuser (106) and Ewing (105). About 50 to 75% of the phosphorus in soybean oil meal is present in the form of phytin (116), and this type of phosphorus is inefficiently utilized by poultry (117). Experiments at Cornell University (118) resulted in the recommendation that chick starting rations should contain at least 0.4% inorganic or available phosphorus. For laying hens the level of inorganic or available phosphorus found desirable by the Cornell investigators (119) was 0.5%, whereas O'Rourke and co-workers (120) found that egg production was supported over a 30-week period by feeding to laying hens rations which contained about 0.43% phosphorus, much of which was in phytin form. Other experiments on phosphorus for poultry have been summarized by Ewing (105) and Heuser (106).

Several investigators have reported that soybean oil meal can serve as the sole source of supplementary protein for laying hens if the mineral and vitamin deficiencies are corrected (64, 106). Ingram and co-workers (121) found egg production satisfactory when soybean oil meal served as the sole source of protein in a laying diet composed of purified ingredients.

For reproduction in poultry, however, the replacement of animal products with soybean oil meal results in decreased hatchability and lowered chick quality owing to deficiencies, particularly in riboflavin and vitamin B₁₂. Pantothenic acid content is also borderline on a corn-soybean oil meal type of ration. Kratzer *et al.* (122) reported that turkeys have a rather high requirement for this vitamin, making pantothenic acid fortification desirable in turkey breeding rations. Soybean oil meal is one of the chief sources of folic acid in practical rations (122, 123). The grains, grain by-products, and animal products are very low in content of folic acid; thus soybean oil meal provides most of the folic acid in practical rations.

Consideration should be also given to the vitamin K content of poultry rations which do not contain alfalfa products. Evidence has been

presented by Anderson *et al.* (124) and Frost (125) indicating that blood-clotting times of chicks fed corn-soybean oil meal rations are prolonged, and that addition of vitamin K-active compounds corrects the prolonged blood-clotting time. Hemorrhages in day-old chicks produced by hens fed breeding diets devoid of alfalfa products have been reported by Cravens *et al.* (126).

It has been reported that unheated extracted soybean flakes increase blood-clotting time when fed to chicks (127) and that vitamin K is ineffective in restoring normal blood-clotting times. But normal clotting times resulted when heated soybean oil meals were fed. That purified soybean trypsin inhibitor has marked anticoagulant properties *in vitro* was demonstrated by Tagnon and Soulier (128). Since heating destroys the trypsin inhibitor and this substance appears to be the cause of the anticoagulant properties, it appears that no practical feeding problems are involved.

The presence of saponin in soybean oil meal has been reported, and the similarity of soybean saponins with those of alfalfa has been established (129). Peterson (130) reported the growth inhibition in chicks fed alfalfa to be due to saponins and that cholesterol in cottonseed oil or soy sterols would overcome the growth inhibition of the saponins. It has been reported that heating soy saponins in the presence of the meal destroyed their hemolytic activity but heating the purified saponins did not. Peterson had also reported the alfalfa saponins to be stable to heat. Further work is required to assess the nutritional significance of saponins in soybeans.

This review is indicative of the prominent place that soybean oil meal plays in practical poultry feeding. When properly prepared it is a source of high-quality protein and may serve as the chief source of protein in poultry rations, providing proper consideration is given to mineral and vitamin fortification.

3. Soybean Oil Meal for Swine

Excellent reviews on feeding of soybeans and soybean oil meal to swine have been published by Robison (131) and Morrison (132). Experimental work on feeding soybean oil meal to swine has closely paralleled that previously outlined with poultry. Much of the earlier work is not directly applicable today because of the mineral and vitamin deficiencies existing in many of the experimental rations employed.

Calcium and phosphorus are the critical minerals when soybean oil meal replaces animal products in swine rations. Vestal (133) reported the results of mineral additions to a corn-soybean ration for hogs, and in later experiments (134) rations containing roasted soybeans and soy-

bean oil meal. Calcium and phosphorus were the only minerals found to be necessary in such rations, and no further improvements were noted from additions of iron, copper, iodine, aluminum, zinc, potassium, manganese, and magnesium. The optimum levels of calcium were reported to be 0.55 to 0.68%, and of phosphorus, 0.40 to 0.45%. Numerous other experiments showing the necessity of mineral supplementation when soybean oil meal replaces animal products in swine rations are reviewed by Morrison (132).

Rations composed largely of corn, soybean oil meal, minerals, and low levels of alfalfa meal are inadequate for brood sows fed under dry lot conditions (135-137). The addition of 10% alfalfa meal or soybean lecithin plus pyridoxine gave normal reproduction, but lactation was not normal and poor growth of the pigs was observed. Fairbanks *et al.* (138) and Krider *et al.* (139) observed similar reproductive failure on all-vegetable rations containing soybean oil meal as the chief source of supplementary protein for gestating-lactating sows. Alfalfa meal, six of the crystalline B vitamins, corn distillers' dried solubles, or a combination of distillers' solubles and alfalfa improved the performance of the sows. In the latter experiment condensed fish solubles were effective in correcting the deficiency or deficiencies of the basal ration. The authors speculated that fish solubles contained unidentified nutrients necessary for gestation and lactation of sows.

For weanling pigs rations composed largely of corn, soybean oil meal, and minerals and six B vitamins were deficient in some essential nutritive factor (140-142). Condensed fish solubles and liver extract were found to be of greatest value in promoting growth.

The unidentified factor required for swine was shown to be vitamin B₁₂ (143), and this vitamin was subsequently shown to be of great value in stimulating growth of pigs fed corn-soybean oil meal (144-146). As with poultry, the discovery of vitamin B₁₂ made possible the addition of the most critical of the unknown factors likely to be deficient in corn-soybean oil meal rations for reproduction and growth of swine.

Numerous experiments on other deficiencies of corn-soybean oil meal rations for swine have been reported (147). According to work at Michigan State College such rations may be low in pantothenic acid. Experiments at Illinois (148) have also indicated that corn-soybean oil meal rations may be low in other vitamins of the B complex.

As with poultry, reports have appeared indicating that there are unidentified factors needed for growing pigs and for optimum reproductive performance of sows (149-151). For swine, however, the response to sources of unidentified factors is definitely marginal; but it is

advisable in utilizing soybean oil meal as the chief source of supplementary protein to recognize a possible deficiency in unidentified factors and to include sources of such possible factors.

Feeding of soybeans to swine will result in soft pork (132); as little as 10% soybean has a decided softening influence on the carcass. In addition to the effect on carcass quality, experiments show conclusively that the protein quality of uncooked soybeans is decidedly inferior to that of soybean oil meal (152). A study (153) of soybean meals heated to different temperatures provides a review of previous work and again demonstrates the value of proper processing in producing a soybean oil meal of maximum nutritive value for swine. Trichloroethylene-extracted soybean oil meal was found to be innocuous to the pig.

Some experiments by Curtin and coworkers (154) are of value in assessing soybean oil meal protein for swine. In these experiments with weanling pigs, soybean oil meal served as the sole source of protein, thus subjecting the protein quality of the meal to a critical test. No improvement in growth occurred on methionine supplementation. In studies by Becker *et al.* (155) soybean oil meal was found to be superior to fish meal when fed in combination with corn, minerals, vitamins, and an antibiotic. Terrill *et al.* (156) found soybean oil meal to be superior to meat and bone scrap as a source of protein in rations for weanling pigs. These experiments indicate that properly processed soybean oil meal is an excellent source of protein for swine. Therefore the chief problem of using it effectively in formulating swine feeds is to include the proper amount and balance of minerals and vitamins.

4. Soybean Oil Meal for Ruminants

a. Beef Cattle

Soybean oil meal is one of the best protein supplements for fattening beef cattle and for the breeding herd. Many experiments in which soybean oil meal has been compared with other high-protein supplements for beef cattle have been reviewed by Morrison (132) and Hayward (64).

The similarity in nutritive value of hydraulic-pressed cottonseed, peanut, and soybean oil meals was reported by Briggs *et al.* (157). Gallup *et al.* (158) studied the comparative value of hydraulic-pressed, screw-pressed, and solvent-extracted cottonseed and soybean oil meals in maintenance and fattening rations for steers and lambs. No differences between meals attributable to the method of processing were evident. The soybean and cottonseed meals were of equal value in promoting nitrogen retention, but soybean oil meal was slightly higher in digestibility of nutrients. Morrison (132) cites eighteen experiments with fattening cattle in which soybean oil meal was compared with cot-

tonseed meal as the supplement. The cattle fed the soybean oil meal required slightly less feed per 100 pounds of gain and sold for slightly more per hundredweight on the average.

Linseed oil meal has proved to be slightly superior to soybean oil meal on the average for rates of gain and for finish of cattle (132). Hinman *et al.* (159) reported the results of feeding tests over a four-year period comparing ground soybeans, soybean oil meal, corn gluten meal, and linseed oil meal when fed with ground corn, corn silage, and mixed hay. Yearling steers weighing approximately 600 pounds were used in the experiments. The steers fed linseed oil meal outgained the steers fed soybeans or soybean oil meal, but all three protein sources were superior to corn gluten meal. There were no significant differences in feed cost or amount of feed per 100 pounds of gain, selling price, or dressing percentages. Those fed soybean oil meal were considered a little firmer in finish, but the cattle fed linseed oil meal had more "bloom." In this test and in others (132), soybeans have been found equal to soybean oil meal for fattening older cattle, but for growing calves soybeans are definitely inferior to soybean oil meal for promoting growth.

The influence of soybean oil meal and complex supplements containing high levels of soybean oil meal on the utilization of low-quality roughages has been the subject of extensive experiments in recent years. Burroughs and Gerlaugh (160) showed that the digestion of dry matter in corn cobs and timothy hay was increased by 14 and 17%, respectively, by the inclusion of soybean oil meal in a low-protein ration for fattening cattle.

Further experiments by Burroughs and co-workers, reviewed by Beeson and Perry (161), have shown that in order for the animal to utilize low-quality roughages the supplement must be fortified with nutritional factors necessary for growth of rumen microorganisms. Beeson and Perry also reported experiments with a supplement which markedly stimulated the growth of steers fed low-quality roughages. It consisted of over 60% soybean oil meal, molasses, bonemeal, salt, and a vitamin A and D concentrate.

Further work on factors necessary for growth of rumen microorganisms has been reported by Bentley *et al.* (162), and reference to other work on this subject was reviewed. It will be of considerable interest and value to learn more of the role that soybean oil meal plays in supplying factors necessary for growth of rumen microorganisms. An excellent review of rumen function and rumen nutrition has been prepared by Moxon and Bentley (163). (See also Chapters 8 and 12.)

The presence of estrogenic substances in soybean oil meal is also of considerable interest in view of work showing that the feeding of diethylstilbestrol to fattening cattle markedly stimulates growth and improves efficiency of feed utilization (164). Genistein, an isoflavone, has been isolated from soybeans by Walter (165) and subsequently shown to be 4.44×10^{-6} times as potent in estrogenic activity as diethylstilbestrol (166). Cheng *et al.* (167) have estimated the estrogenic potency of genistein from soybean oil meal to be 2×10^{-5} times that of diethylstilbestrol.

Further studies will be required to determine the significance of the estrogenic activity of soybean oil meal on growth and reproduction of animals.

b. Sheep

Experimental work dealing with soybeans and soybean products in sheep feeding has been reviewed by Morrison (132).

The effect of heat treatment and oil extraction on the digestibility and utilization of soybean protein has been studied by Miller and Morrison (168). A low-protein basal ration (about 1%) was used, and the soybean products were added to supply an additional 10% of protein. Apparent digestibility averaged 62.9% for the raw soybean ration; 69.0% for the ration containing light-colored, 44% protein, extracted soybean oil meal; and 70.6% for the ration containing heat-treated, 44% protein, soybean oil meal. Addition of soybean oil to the soybean oil meal ration to equal that of the oil in a soybean ration resulted in an average digestibility of 71.1%, compared to 73.1% for a ration without the added oil and 64.3% for the raw soybean ration. Nitrogen storage averaged 18.2% for the raw soybean ration; 23.7% for the light-colored, 44% protein, extracted soybean oil meal ration; and 26.3% for the heat-treated, 44% protein, extracted soybean oil meal ration. Addition of fat did not influence nitrogen storage. These authors concluded that higher digestibility and not greater efficiency of digested nitrogen accounted for the greater value of the heated soybean oil meal protein.

Nitrogen balance experiments by Cornell workers (169, 170) comparing soybean oil meal, linseed oil meal, and corn gluten meal indicated a slight advantage of soybean oil meal in the first series of experiments, but in the second series all three supplements were found to have equal value. Practically identical results for cottonseed and soybean oil meal for lambs were obtained by Briggs *et al.* (171), but peanut meal was definitely inferior.

The replacement of part of the oil meals by urea in rations for growing lambs has been a subject of considerable study. Johnson *et al.* (172) found urea to be effective as a nitrogenous supplement to a ration containing approximately 6% crude protein so long as the protein equivalent supplied by urea did not raise the total protein equivalent above 12%. In seventeen of twenty-one comparisons made, the nitrogen of the soybean oil meal ration was better utilized than that of the urea ration. Loosli and Harris (173) reported that the addition of methionine to rations containing urea improved nitrogen retention of lambs. Block and Stekol (174) showed that inorganic sulfur was utilized by ruminants and converted into wool and milk proteins. Several papers on sulfur in sheep nutrition have been published, the latest (175) indicates that rations containing soybean oil meal were superior for gains and wool growth to those in which two-thirds of the nitrogen of the soybean oil meal was replaced with urea nitrogen. The addition of sulfur to the urea rations

resulted in a marked improvement, and results thus obtained were comparable to those on the soybean oil meal rations.

A study by Slen and Whiting (176) of proteins varying widely in sulfur amino acid content failed to demonstrate a correlation between these amino acids and performance of mature range ewes for lamb and wool production. Urea was inferior to soybean oil meal, linseed meal, field peas, alfalfa meal, lactalbumin, and meat meal for lamb and wool production, and addition of sulfur with the urea was of no advantage. The protein under study contributed approximately 40% of the protein in the ration (7% protein rations) until 6 weeks before lambing and 60% thereafter (1.1% protein rations). There were differences between the above supplements for lamb and wool production. Soybean oil meal, linseed meal, and alfalfa meal were of equal value for lamb and wool production. Peas were inferior in one experiment but not in the second.

Noble *et al.* (177) studied the value of urea and soybean oil meal, alone and in combination with methionine in low-protein fattening rations for lambs. Neither methionine nor urea improved gains, but a combination of the two increased slightly the rate of gain. Additions of soybean oil meal to the basal ration consistently improved rate of gain and feed efficiency. Methionine added to the ration supplemented with soybean oil meal was without effect.

Soybean oil meal is an excellent protein supplement for sheep. Some differences between protein supplements have been reported, and digestibility of soybean oil meal for lambs has been found to be superior to that of whole soybeans. Soybean oil meal has given more consistent results than urea-containing feeds. (See also Chapter 12.)

c. Dairy Cattle

The value of soybean oil meal in calf starter rations has been reviewed by Hayward (64). Stein and co-workers (178) indicate that solvent-extracted soybean flour can be used to replace up to 43% of the non-fat dry milk solids in a milk-replacer formula for dairy calves. Higher amounts depressed appetite, retarded growth, and resulted in a poor appearance of the calves. Corn, cottonseed, and soybean oils fed to calves (179) result in digestive upsets and high mortality, whereas hydrogenated soybean oil can serve as the source of fat for young calves (180). This would indicate that certain fatty acids are responsible for the effect of these oils on the young calf.

Numerous feeding trials have been reported which deal with the use of ground soybeans and soybean oil meal in the feeding of dairy cows. Many of these have been reviewed by Morrison (132) and Hayward (64). In general the data show that soybean oil meal is an excellent protein source for dairy cattle. It has also been demonstrated that ground soybeans will serve as a source of protein for dairy cows but are not so well liked and depress the vitamin A and yellow color of butter-

fat (181, 182). This vitamin A- and color-depressing factor is found primarily in the oil, but a small amount was present in the meal after extraction of the oil (183, 184). It has also been demonstrated that the feeding of ground soybeans causes the butterfat to become soft owing to the unsaturated fatty acids present in the oil (185). Plasma concentration of vitamin A and carotene in calves is also depressed by raw soybeans (186).

One important problem involving extracted soybean oil meal in dairy feeding is the effect of the fat level in the grain mixture on milk and butterfat production. The increased demand in past years for fats for human consumption has resulted in the development of more efficient methods of extracting fats from the oil-bearing seeds with the result that the oil meals are much lower in fat than formerly. Hence the grain mixtures containing extracted oil meals for dairy cattle feeding are lower in fat than formerly. The effect of fat level in the concentrate fed to dairy cattle has been summarized by Monroe (187).

The extensive trials conducted at Cornell have been summarized by Loosli (188, 189). These results show a 4.1% increase in 4% milk by using the higher levels of fat in the grain mixtures; the range in fat levels varied from a low of 0.7% to a high of 10%, and in terms of 4% milk the difference amounted to 1.4 pounds daily. Within the normal levels of practical feeding (2.0 to 5.9%) the difference amounted to 0.8 of a pound of 4% milk daily. Loosli states: "Thus, the question appears to be purely an economic one as to whether the fat is worth more than the extra feed." Extra amounts of hay had been shown by the Cornell workers to minimize the differences between moderately low and high fat rations.

Monroe (187), after summarizing the work on fat levels in dairy feeds, states: "Other work in which practical grain mixtures have been used in connection with the liberal feeding of hay has failed to show any significant difference in production with grain mixtures varying from a low of 2.7% fat to a high of 4.9%.

"Efficient production can be obtained on low fat (2.5%) mixtures. There is no evidence to indicate that low fat grain mixtures adversely affect the health or general welfare of milking cows. Thus the answer to the question of how much fat the dairy grain mixture should contain is based on the relative cost of fat as compared to other nutrients."

The feeding of trichloroethylene-extracted soybean oil meal to cattle results in a fatal hemorrhagic, aplastic anemia (190). It has also been reported to be toxic to sheep, although the aplastic anemia syndrome observed in cattle did not develop (191). Attempts to determine the toxic factors involved in trichloroethylene-extracted soybean oil

meal have been unsuccessful (192), although some evidence was presented indicating that the soybean per se may not be necessary for the trichloroethylene toxicity. (See also Chapter 6.) *

d. Soybean Oil Meal for Dogs

Few studies have been published on soybean oil meal in feeds for dogs, although this ingredient is commonly used in such rations. Leluo (193) concluded that dogs can digest and use about two-thirds of the protein of soybeans, peas, and rye in contrast to 85 to 95% of milk proteins. McCay (194) states that he has used "substantially more" than 5 to 10% of soybean oil meal in formulas for kennel feeding without difficulty. In 1949 McCay stated: "Decisive trials have never been made to see whether plant products such as soy meal can replace meat products entirely throughout the span of life or even the life cycle, growth, reproduction, and lactation of the dog." It would appear that soybean oil meal could be used extensively in dog food formulas at the expense of products of animal origin, providing the mineral and vitamin fortification is adequate. This is on the assumption that the balance of amino acids in soybean oil meal meets the needs of the dog, as it does for other animals with simple stomachs.

Some data are now available dealing with the use of simplified all-vegetable rations for dogs. Campbell and Phillips (195) used a basal diet composed of corn, soybean oil meal, alfalfa meal, salt mixture, cottonseed oil, vitamins A and D, and niacin. Weanling pups were fed the basal ration and rations supplemented with vitamin B₁₂, fish solubles, brewers' yeast, and fresh beef liver. Growth was comparable in all groups, and the authors concluded that the simplified basal diet furnished all the nutritional factors necessary for growth and maintenance. The basal diet, however, was deficient in some factor necessary for reproduction and lactation. From these results we can conclude that soybean oil meal is an excellent source of protein for the dog, but we can only assume by analogy that the processing factors affecting protein quality of soybean oil meal for poultry and swine are applicable to dogs as well.

VII. FUTURE TRENDS IN SOYBEAN OIL MEAL UTILIZATION

The future potential application of soybean oil meal and other soybean products would, by all indications, appear to grow. Certainly the

* Reaction of cysteine with trichloroethylene resulted in synthesis of S-(dichlorovinyl)-L-cysteine which produces fatal aplastic anemia on oral administration to calves. It is suggested that a compound of this type may be formed during extraction of soybeans with trichloroethylene. L. L. McKinney, F. B. Weakley, A. C. Eldridge, R. E. Campbell, J. C. Cowan, J. C. Picken, Jr., and H. E. Biester, *J. Am. Chem. Soc.* **79**, 3932 (1957).

trend over the past several decades has been toward the greater inclusion of soybean oil meal in livestock and poultry feeding. Basic nutritional research, research directly involving soybean oil meal in feeding, and technical advances in processing soybean oil meal to bring out the inherent quality of the protein have been responsible for the great reliance placed on soybean oil meal in formulating top-quality rations for all classes of animals. As further basic nutritional research elucidates the now unknown growth factors and technical improvements in processing are made, it seems likely that more soybean oil meal will be used. Further chemical research will no doubt make available economically pure compounds of nutritional importance such as vitamins and amino acids which are needed when great dependence is placed on soybean oil meal in formulating rations. All these developments will make possible greater usage of soybean oil meal in nutrition.

The ever-increasing population in the United States would seem to indicate a substantial increase in livestock numbers. It has been estimated that a soybean crop of 500 million bushels would be required to make up the deficit of protein feeds required to feed properly all farm animals (196). Hafner (197), in comparing the total oilseed meal consumption in the United States with the number of animal units fed, came up with the following figures: for the crop year 1938-39, 40 million grain-consuming animal units consumed 1 million tons of oilseed meals; by 1950, because of the fast-expanding oilseed production, this ratio had decreased to 20:1.

Hafner's figures indicate that by 1975 the number of animal units fed in the United States will increase to 220 million as a direct result of the population increase. Thus, at the present 20:1 ratio, the 220 million grain-consuming animals will need 11 million tons of oilseed meals. But the ratio does not seem to be leveling off yet, and indications are that by 1975 it will have reached 15:1. This would indicate that, by 1975, 220 million grain-consuming animals will have to be fed 14.7 million tons of oilseed meals, a 70% increase over the amount used in the 1953-54 crop year. Any appreciable increase in oilseed consumption would come from an increase in soybean production, as the rapid expansion of the industry in recent years would indicate. Hafner estimates that 11.2 million tons of soybean oil meal will be fed to animals by 1975 from the total of 14.7 million tons of oilseed meals. This is roughly equivalent to a soybean crush of 450 million bushels. At this projected rate of development, the industry's annual capacity of around 300 million bushels would be adequate until 1960.

A higher standard of living and increased knowledge favor the widespread use of soybean oil meal, but certain other factors tend to depress its utilization. Some of these are: development of oilseed crops

competitive to soybeans, expanded use of urea in livestock feeding, and any possible acreage restrictions. The bulk of the evidence is, however, indicative of an expanding need for soybean oil meal.

Besides the increasing demand in the United States, there are indications of a probable expansion in world use of soybean oil meal. The big question in the world picture, however, is the volume of Manchurian soybeans that may enter the market. There have been reports of a growing trade by Japan and Western Europe with China. At present the full weight of this trend is not felt by the United States because much of this volume moves directly to the U.S.S.R., where soybeans are covered by a Chinese-Soviet trade agreement (198). Should the economic status of the Far Eastern countries continue to improve, soybeans and soybean oil meal produced in the United States will find a sizeable market there.

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CHAPTER 15

EDIBLE ISOLATED SOYBEAN PROTEIN

S. J. CIRCLE WITH D. W. JOHNSON

I. INTRODUCTION

One compelling fact emerges from the many discussions on human dietary requirements throughout this volume: the need for ever-increasing amounts of nutritionally adequate protein to feed the growing world population. Traditionally, animals have been looked to as the most desirable source of high-quality palatable protein; however, the supplies of high-grade animal protein are not excessive, and indeed the majority of the world's population has not even regained the already inadequate prewar levels of per capita per day protein supplies (1-3).

The population of the world in 1956 was increasing at the rate of 43 million persons per year (4). Despite an annual increase in gross total supplies of protein, the critical ratio of protein supplies to population was falling off (5-14). In view of the unfavorable ratio of conversion of vegetable proteins to animal proteins by feeding (10, 14-17), this shortage in high-quality protein cannot be met entirely by an increase in animal production (see Chapters 1 and 9), and more reliance than ever will have to be placed on plant sources of protein. There will be a significant place in the world food economy for the herbivorous ruminants, who fulfill a useful function in converting roughage and forage otherwise unsuitable for man (14), but per capita production of non-ruminant animals will probably decrease.

Plant protein is not *a priori* poor protein, and animal protein good protein (6, 18-21). (See also Chapter 1.) There are use limitations to plant protein sources, but these are not limitations necessarily of their protein. Oilseeds as such are not suitable for ingestion as a major source of human or animal dietary protein because of the large quantities of fat which accompany the protein; processed oilseed meals with decreased fat content are more suitable. For high-energy rations, it is necessary mechanically to "refine" the meals by removal of as much fiber as possible; soybean meals containing at least 50% protein are now the usual product for broiler rations. For human beings and young mammals it is advantageous chemically to refine soy meal further into isolated soy protein freed from the insoluble fiber, soluble carbohydrates, and other non-protein material in the meal.

The soybean as a world crop in 1956 was estimated to total 854 million bushels (22), with 456 million bushels representing the United States crop

(23). Soybeans as a technical raw material stand near the top as a source of isolated vegetable protein, in respect to quantity as well as quality.

The isolation and properties of proteins from plant sources were reviewed in Chapter 10. Anson (Chapter 11) pointed out the advantages of isolated plant proteins, particularly isolated soybean protein, as a human foodstuff, and predicted that a technology will be developed to produce new protein products from vegetable sources which will be acceptable and will fulfill dietary needs. Some aspects of this technology have already appeared in the literature (24). In this chapter we shall document the continuing growth of the edible isolated soybean protein industry in the United States (still in its infancy), describe the properties of edible isolated soybean protein, and suggest methods of formulating it into familiar and palatable food forms.

II. ISOLATED SOY PROTEIN COMPARED TO SOY FLOUR

Soybean protein in the form of mechanically refined soybean flour is already in extensive use as human food (24) (see also Chapter 9). As pointed out in Chapter 10, industrial grades of isolated soybean protein compete with mechanically refined soybean flour in several applications, despite their higher price, because of their superiority with regard to fiber content, color, and adhesive strength. Solvent-extracted soybean flakes are the raw material for both the soy flour (dry-processed) and the protein isolate (wet-processed); however, the isolate is essentially all protein, whereas the flour is only half protein. A sharp distinction exists between the two (25). In this chapter the word *protein* is reserved for the isolate, as defined by Smith in Chapter 10.

The edible isolated soybean protein is generally superior, compared to the mechanically refined flour, in flavor, color, texture, fiber content, viscosity and gelling properties, ease and versatility of use, and nutritional adequacy. The isolate is physiologically more completely assimilable, being practically free of fiber and free of inhibitory and other factors found in improperly processed flour or meal (26–28).

Soy flour will continue to be used in foods, if for no other reason than its lower price, which is at least one-fourth to one-fifth that of the isolate. In most of the food applications listed in Section VI, however, the use of the isolate results in much better products compared to those made with flour; indeed some applications are possible only with soy protein isolate.

III. AVAILABILITY AND FORMS OF EDIBLE SOY PROTEIN ISOLATE

Several types of edible isolated soybean protein as opposed to mechanically refined soy flour have already appeared in the United States

market in both commercial quantities and pilot amounts. They range in price from 28 to 90 cents per pound (1957). The only other highly concentrated vegetable protein sold in the United States for edible purposes is wheat gluten; its price is about 30 cents per pound (1957).

No attempt will be made to review in detail methods of manufacture of isolated soybean protein; a comprehensive survey of this subject and other information of interest through 1949 is available (29, 30), and more recent work has been described in Chapters 10 and 11 and by Anson and Pader (31). The solvent-extracted soybean flour or flake is extracted with an aqueous alkaline solution, the insoluble residue is removed, and the protein is precipitated from the screened or clarified extract by food grade acid to form "curd" and "whey." The spent insoluble residue is not generally used for food but has been used as an ingredient of ruminant feeds and for special applications.

1. Soy Protein and Soy Proteinate

The unhydrolyzed isolate is available in two forms: *protein* (unmodified, unhydrolyzed isolate, insoluble in water, and having a *pH* of 4.5 to 5.0 in water), and *proteinate* (unhydrolyzed isolate, neutralized to a point where it is directly dispersible in water, and having a *pH* preferably in the range 6.5 to 7.5 in water). Protein is usually separated from "whey" in the form of a curd cake, and dried in a forced-air dryer (Chapter 10). Proteinate is usually prepared from the curd by neutralizing to a *pH* range of 6.5 to 7.5 or higher, and drying on a roller dryer or in a spray dryer (24). It is inherently somewhat more expensive to manufacture than protein.

Smith (page 258) states that under laboratory conditions the yield of isolate may be as much as 42% of the weight of oil-free meal; however, he considers a yield of 30% under commercial operations to be good. The problems of disposal of spent insoluble residue and of "whey" remain a challenge. On the conservative assumption of 50 pounds of clean soybeans per bushel and 22% yield of soy protein isolate based on raw bean weight, the 1956 United States soybean crop of 456 million bushels (23) would afford more than 5 billion pounds of edible isolated soy protein, if all of it were converted.

Typical analyses of commercial soy protein and proteinate are given in Table I.

2. Modified Forms of Soy Protein Isolate

There are also available modified, hydrolyzed, isolated soy protein products, about which there has arisen some confusion. Soy protein mildly hydrolyzed with alkali (32) is used in many industrial appli-

cations (29), mainly for its adhesive properties. It is not recommended for food uses, since its nutritional value has been damaged to some extent, and its physical properties adversely modified for some food applications. (See also Chapter 10.)

The enzyme- and acid-hydrolyzed types (24) are usually extensively hydrolyzed and can no longer be considered proteins. The enzyme-hydrolyzed soybean protein products are sometimes referred to by the misnomer soy albumen, although they are mainly polypeptides;

TABLE I
TYPICAL ANALYSIS OF COMMERCIAL ISOLATED SOYBEAN PROTEIN

Product	Composition			
	Protein ^{a,b}	Moisture	Ash ^b	Fat ^b
	%	%	%	%
Protein ^c	>90	9.0	2.2	<0.1
Protein ^d	>90	9.5 ± 1.0	2.5	<0.5
Protein ^e	>90	6.0	6.0	<0.1
Protein ^e	>90	5.0	7.0	—

^a N × 6.25.
^b Moisture-free basis.
^c The Glidden Co., Chicago, Illinois.
^d The Drackett Products Co., Cincinnati, Ohio.
^e Gunther Products, Inc., Galesburg, Illinois.

they are employed in food products chiefly for their whipping properties. They have, however, some disadvantage flavorwise unless used in relatively low concentration. The acid-hydrolyzed types are mixtures of peptides and amino acids high in content of monosodium glutamate, have meat-like flavors, and are used mainly as condiments; the latter must compete in price with similar products made from corn and wheat glutens.

3. Partial Isolates of Soy Protein and By-Products

A bland-tasting product called Gelsoy, which is essentially the spray-dried aqueous extract of an ethanol-washed soybean flour, is described in Chapter 10. It may be considered equivalent to dry soybean “milk” (with some alcohol-soluble factors removed) or to a mixture of isolated soy protein with the associated “whey” not removed. Since it is made by wet extraction, its cost of manufacture based on protein content will approach that of the isolate. Such a product could be readily manufactured in any plant equipped to make the neutral soy protein

which is free of "whey" and should be somewhat cheaper in price than the proteinate on a protein basis.

IV. PROPERTIES OF UNMODIFIED ISOLATED SOY PROTEIN

Emphasis will be placed on the physical and chemical properties as they affect the use of soybean protein in foods (24), either in the form of the insoluble, isoelectric protein, or in the form of soluble, neutralized proteinate. Other physical and chemical properties are reviewed elsewhere (30).

1. Methods of Dispersing Protein and Proteinate

Soy protein is not dispersible in water without the addition of alkali. Since it can be dried in granular form, it can be ground to, and is available in, different mesh sizes. When immersed in water, it will absorb three to four times its weight and will come to a *pH* in the range of 4.5 to 5. On further treatment with alkaline reagents of food grade such as sodium carbonate, trisodium phosphate, or sodium hydroxide, the protein can be made to disperse at a *pH* above 6.5. In some instances it may be desirable to reach a *pH* above 8 to achieve complete dispersion; readjustment to the *pH* range 6.5 to 7.5 may then be made by the addition of an acid of food grade. With proper manipulation, weaker alkaline reagents such as sodium bicarbonate and disodium hydrogen phosphate may be used with less water to form dough-like plastic masses having properties of extensibility, cohesiveness, and elasticity, and baking qualities approaching those of wheat gluten doughs. The judicious use of heat in the lower temperature range below 60° to 70° may assist greatly in hastening the dispersion process.

Soy proteinate is usually dispersible in water without the addition of any agent.

Soy protein dispersions, when properly made and incorporated into many food formulations, impart properties such as dough-forming, moisture-holding, fat-binding, emulsifying, foaming, film-forming, thickening, stabilizing, gelling, cohesiveness, and adhesiveness.

2. Viscosity Behavior. Effect of High Temperature

Soybean protein can be dispersed in concentrations in the range of 8 to 10% without undue thickening; however, above this concentration range the viscosity is sensitive to changes in *pH*, temperature, or chemical environment, and with sufficient increase in temperature, such dispersions can be made to gel (29). Autoclave temperatures are especially effective for gelling. (See page 280.)

Unmodified soybean protein contains disulfide linkages which can

be split by the action of sulfhydryl and sulfite groups to exert a thinning action on the viscosity. The viscosity can be further modified by hydrolysis, either acidic, alkaline, or enzymatic.

3. Flavor

The flavor of edible isolated soybean protein, when carefully made, is essentially bland. Among the factors which may affect the flavor are degree of steam treatment given the source material, degree of separation of non-protein constituents of the source material, presence of iron and copper salts, acid used in precipitating, and temperatures and *pH* sustained during production of the isolate.

The flavor and texture can be modified by the techniques used in converting the isolate into various food forms, and by the type of cooking or other treatment employed during manufacture of converted finished products and their preparation for consumption. Of course, flavor can be still further modified by the addition of various flavoring materials.

V. NUTRITIONAL VALUE OF EDIBLE ISOLATED SOYBEAN PROTEIN

1. Nutritional Value for Human Beings and Animals

a. Isolated Soy Protein

Until about 1948 the only commercially available isolated soybean protein was of the alkali-hydrolyzed type (32). Although it was not recommended for nutritional use, nevertheless several nutritionists (33–35) employed it in their studies, since it was the only isolated protein which was readily available. When supplemented with methionine, this isolated soy protein served as a basis for a diet in which the protein was of predominantly vegetable origin. Such a diet could be made adequate for the growth of the rat, rabbit, chick, pig, and monkey, and has been helpful in research on several nutrition problems. There is evidence that heat is not required to improve the nutritional quality of the isolate, as is needed for soybean meal or flour (28, 33). (See also Chapters 11 and 14.)

More recently pilot and commercial quantities of soy protein and proteinate have become available, and have been evaluated by nutritionists (36–47). In one of these studies (37), the soy protein was exhaustively extracted with boiling water and with 70% isopropyl alcohol before feeding. It was adequate as sole source of protein for reproduction and weaning in five generations of rats. In another study, the soy protein fed to lambs gave a biological value of 82.4% compared with 72.7% for milk casein and 83.1% for blood fibrin (38).

In a clinical study over a period of four years, Szujewski (48) fed 8 to 10 grams of soy protein daily to each of over 200 persons, patients with a variety of illnesses. Although the age group covered the range from infants to quite elderly people, no untoward reactions were observed, and all patients tolerated the soy protein well. Objective findings in all groups could be described as clinical improvement in color of skin, and in tissue turgor of the skin and of mucous membranes of the mouth.

b. Soybean Oil Meal

The literature on the nutritional value of soybean oil meal is truly voluminous. This is reviewed in Chapters 5 and 14. It is now generally accepted that soybean meal, when properly heat-treated to inactivate suppressive factors and to improve digestibility, and when properly supplemented with methionine, vitamins, and known and unidentified growth factors, is nutritionally adequate as chief or sole protein source for the growing rat, chick, and pig (21, 49, 50). It is also used widely as the protein mainstay of many dog and cat foods.

c. Soybean Milk, Soybean Curd, and Oriental Products

As pointed out in Chapter 11, isolated soybean protein in the form of soybean curd (unfermented) and in the form of various other fermented and unfermented products from the soybean has been eaten by large populations for thousands of years in the Orient (see also 51, 52). In the preparation of bean "curd" or "cheese," soybeans are first processed into the form of a "milk" (53). An extensive literature which has accumulated on experience in the feeding of soybean milk and curd to human beings has shown that it can substitute nutritionally for bovine milk and cheese (49, 51-57). A good deal of this feeding has been prescribed for human infants in cases of allergy to bovine milk. Soybean milk has also been fed to convalescent and normal adult humans (49, 54, 55). The human experience is confirmed by other studies and is substantiated by experiments with the rat and calf (58-73; see also Chapter 9).

Two types of soybean milk have been employed, one made from a fiber-free water extract of soybeans or soybean meal, and the other from a suspension of soybean flour in water, which still retains the fiber; the former is claimed to be superior (57).

2. Amino Acid Composition

Considerable data have been accumulated on amino acid composition of soybeans, soybean meal and flour, and soybean protein (18, 30,

50, 74-78). Data on values of amino acid composition for unhydrolyzed isolated soybean protein and for soybean flour or meal are compared with values for beef muscle, fish muscle, whole egg protein, and milk proteins in Table II. The comparison shows that there is little difference between the amino acid analysis of the isolate and that of the

TABLE II
COMPARISON OF THE AMINO ACID COMPOSITION OF ISOLATED SOYBEAN PROTEIN WITH THE PROTEIN OF SOYBEAN FLOUR AND WITH CERTAIN ANIMAL PROTEINS

Amino acid	Protein product							
	Isolated soybean protein		Soybean flour	Casein	Lactalbumin	Beef muscle	Fish muscle	Whole egg
	(grams per 16 g. of nitrogen)							
Arginine	8.2	8.3	7.3	4.1	3.6	6.5	5.5	6.6
Cystine	0.7	0.6	1.8	0.4	3.7	1.3	1.3	2.3
Histidine	2.6	2.6	2.4	2.9	1.9	3.5	—	2.4
Isoleucine	5.8	6.5	5.3	6.4	6.5	5.3	5.1	6.8
Leucine	8.4	7.5	7.7	10.0	14.3	8.2	7.6	9.0
Lysine	6.0	6.8	6.3	8.0	9.3	8.6	8.8	6.3
Methionine	1.4	1.0	1.4	3.0	2.0	2.5	2.9	3.1
Phenylalanine	5.8	5.0	4.9	5.3	4.0	4.1	3.7	5.9
Threonine	4.0	3.9	3.9	4.4	5.2	4.4	4.5	5.0
Tryptophan	1.1	1.0	1.4	1.4	2.4	1.2	1.0	1.7
Tyrosine	—	3.4	3.2	5.9	4.1	3.4	2.5	4.4
Valine	5.8	5.5	5.2	7.2	5.5	5.5	5.2	7.4
References	a	b	c	c	c	c	c	c

^a "Technical Literature." The Glidden Co., Chicago, Illinois.

^b "Technical Bulletin." The Drackett Products Co., Cincinnati, Ohio.

^c M. L. Orr and B. K. Watt, *U.S. Dept. Agr., Agr. Research Service FE-101*, preliminary draft (1955); *Ibid.*, *Home Economics Research Rept.* 4 (1957).

meal. The limiting amino acid is methionine, and, aside from this, soy protein isolate appears to be adequate in its content of the essential amino acids.

3. Supplementation

The methionine deficiency of isolated soybean protein can be remedied by mutual supplementation with proteins from other sources,

or by addition of synthetic DL-methionine. Soybean protein presumably is adequate for the rat with respect to lysine content, since addition of lysine did not improve the performance of the diet (79).

a. Mutual Supplementation

It is now an axiom in protein nutrition that a better balance of amino acids can be achieved by a judicious mixture of two or more protein sources than from a single protein source, and with better economy (21). The addition of relatively small percentages of soy flour to wheat flour gave a marked improvement in growth of rats over that obtained with wheat flour alone, whether in baked form or unbaked (80-82). A similar enhancement holds for supplementation of corn flour (83), rice (84, 85), and sesame meal (21, 86; see also Chapter 18).

b. Isolated Soy Protein As a Source of Lysine

Isolated soy protein offers a good source of essential amino acids to upgrade the nutritive value of other vegetable proteins. Over 1 pound of lysine is contained in 20 pounds of commercial isolated edible soybean protein. Based on a quotation of 28 cents per pound (46), the price of this pound of lysine would be \$5.60, but some 17 pounds of other valuable amino acids would be supplied without extra cost. This is a major consideration affecting the economics of supplementation with lysine and has a bearing on the use of synthetic lysine. Although L-lysine in soybean protein is at present cheaper than its synthetic counterpart, there may be other factors which should be considered in deciding on the proper approach to supplementation. (See Chapter 13.)

VI. FOOD USES OF EDIBLE ISOLATED SOY PROTEIN

Burnett (24) has reviewed the literature through 1950 on food products from soybean protein. There has been considerable interest in soybean flour, but little has been done with unmodified, unhydrolyzed isolated soybean protein or proteinate. In the interim little more has been published.

The authors favor the premise that ready acceptance of the soy protein isolate in the diet will be greatly facilitated by its presentation in the form of indigenous familiar foods, both in the United States and in other countries. This does not rule out the possibility of new and unique food forms not extant today.

The discussion which follows is partially in the nature of an extension and expansion of the discussion in Chapter 11 on modern possibilities of utilizing isolated soy protein in foods. For the most part this

developing field is uncharted. Hence, some of the statements are based of necessity on the unpublished work of the authors; included are representative food formulations from the authors' laboratory.*

The food types to be discussed are those familiar ones which, in the authors' opinion, lend themselves most feasibly to fortification, supplementation, or replacement, partial or complete, of their usual protein content by isolated soybean protein.

Methods of dispersing soy protein have been discussed in Section IV. In many applications in which a dry product is indicated, the water-dispersible neutral proteinate can be incorporated by dry-blending. In other applications, a water dispersion of the protein is the preferred form, with subsequent drying if desired. In still other applications, the insoluble isoelectric type of protein can be used directly, with or without addition of sufficient sodium bicarbonate, disodium phosphate, or other alkaline reagents to neutralize its acidity; it can be added dry or with preliminary soaking in water. Heat may or may not be used to aid the process of dispersion. In general, the manipulative technique is chosen for or dictated by the type of product in mind. The formulations given below are suggestive and may be modified as desired.

It is obvious that a similar approach may be made for all countries, based on their indigenous diets; soybean protein can probably be offered in the form of familiar foods, thus promoting acceptability.

1. Dairy-Type Products

These dairy-type products include milk, creams of varying fat content, whipping cream, whipped topping (24, 87), sour cream, ice cream and frozen desserts (24), cream cheese, cottage cheese, cured cheeses, margarine (88), spreads, yogurt, fortified milk and flavored drinks, puddings, and dry powders. Edible isolated soy protein can be incorporated into any of these products as an addition to milk protein for the purpose of protein fortification, or it may be used for replacement of milk protein where permitted by law. Since most dairy products are emulsions containing fat, protein, carbohydrate, and minor constituents, these products may be simulated by appropriate techniques, with all these constituents replaced in whole or in part by their counterparts from vegetable sources. The flavor of dairy products can be fairly closely simulated in most of the items above, with the possible ex-

* Acknowledgment is made of the collaboration of S. S. Frank and R. W. Whitney of The Glidden Company, Central Organic Research Laboratory, Chicago, Illinois, in devising these formulations.

ception of fluid milk. A considerable amount of work has been done on margarine flavors, butter flavors, and the flavor of fresh bovine fluid milk. This experience should be helpful as applied to soybean milk.

All-Vegetable Coffee "Cream"

17.5 g. soy protein	15 g. dextrose
250 g. water	5 g. citric acid
5% solution trisodium phosphate	1.25 g. calcium phosphate, dibasic
5% solution citric acid	Additional water for final
98 g. hydrogenated vegetable oil	make-up
2 g. mixed mono- and diglycerides	

Combine water and soy protein, heat to 150°F., and add enough 5% trisodium phosphate to dissolve the protein with constant stirring at a pH of approximately 8.5. Clarify by filtering or centrifuging if desired. Add enough citric acid solution slowly with stirring to bring back to pH 7.0. Add remaining ingredients and enough water to make 500 g. of mixture, pasteurize at 150°F. for 30 minutes, homogenize, and cool immediately. Use in hot coffee like any other cream.

All-Vegetable Whipped Topping

122.5 g. hydrogenated vegetable oil	1 g. spray-dried vanilla flavor
2.5 g. mixed mono- and diglycerides	50 g. sucrose
	1 g. anhydrous sodium bicarbonate
17.5 g. soy protein	307 g. boiling water

Mix together the vegetable oil, mono- and diglyceride mixture, soy protein, vanilla, sucrose, and baking soda, and store. When ready to use, place entire mixture into a fountain-type mixer, add boiling water, stir for 2 minutes at low speed, and for 3 minutes at high speed. Fill a gas dispenser, cool thoroughly, and then charge the dispenser with gas cartridge. Cool again. Serve as desired, as a topping for desserts.

All-Vegetable "Cream Cheese"

250 g. water	40 g. soy proteinate
165 g. hydrogenated vegetable oil	1.2 g. salt
USP conc. lactic acid	

Mix water, vegetable oil, soy proteinate, and salt, pasteurize at 160°F. for 30 minutes, and homogenize. Add enough concentrated lactic acid to adjust to pH 5.4. Cool. Shape into bricks, and wrap in aluminum foil.

Non-Milk Chocolate Frozen Dessert

20.0 g. cocoa syrup	2.0 g. gelatin
40.0 g. dextrose	50.0 g. sucrose
50.0 g. hydrogenated vegetable oil	320.0 g. water
22.5 g. soy proteinate	5.0 g. vanilla extract

Combine all ingredients but the vanilla extract, pasteurize at 160°F. for 30 minutes, homogenize, and cool. After the mixture has been thoroughly chilled, add vanilla and freeze.

All-Vegetable High-Protein Chocolate Drink

25.0 g. soy proteinate	350.0 g. water
25.0 g. sucrose	5.0 g. vanilla extract
100.0 g. cocoa syrup	

Blend soy proteinate and sugar, and then combine all ingredients. Pasteurize at 160°F. for 30 minutes, and cool. Serve as desired.

All-Vegetable High-Protein Non-Starch Chocolate Pudding

14.0 g. chocolate liquor	15.0 g. lactose
72.0 g. sucrose	355.0 g. hot water
3.2 g. salt	5.0 g. vanilla extract
60.0 g. soy proteinate	

Melt chocolate liquor. Sift dry ingredients together. Combine all ingredients in blender or by mechanical stirring. Fill pudding dishes, and cool. Serve with all-vegetable whipped topping.

2. Meat-Type Products

Anson (Chapter 11) has discussed the possibility of manufacturing meat-like products of fibrous structure from soybean protein; see also Dudman (89). The manufacture of ground meat-type products is less complicated. Included in the latter are sausages, meat loaves, ground meats of various kinds (hamburgers and patties), frankfurters, and baby meats. Soy protein also offers promise as an extender or partial substitute for gelatin, egg, and fish protein products, and as a meat binder.

Graded, coarsely ground granular soy protein is used to obtain desirable texture, bite, and mouth feel in the "meat loaf" formulation. This can be treated in various ways to maintain granularity even after cooking. By use of calcium hydroxide, and a *pH* below 5.5, a relatively tough bite is obtained. By use of trisodium phosphate at a slightly higher *pH* (5.5 to 6), a more tender bite is achieved. The finely-ground soy protein is dispersed at a still higher *pH* (6.5 to 7.5) to act as binder.

All-Vegetable "Meat Loaf"

112.5 g. granular soy protein	2-3 drops 10% red food color
12.5 g. finely-ground soy protein	12.5 g. hydrogenated vegetable fat
350.0 g. water	5.0 g. hydrolyzed vegetable protein
3.1 g. calcium hydroxide	2.5 g. monosodium glutamate
10.0 g. trisodium phosphate	5.0 g. seasoning
5 ml. 10% sodium hydroxide	

Combine 12.5 g. of granular soy protein, calcium hydroxide, and 50 g. of water, and let stand for 1 hour at 120°F. At the same time combine 100 g. of granular soy protein, trisodium phosphate, 250 g. of water, and the red food color (certified food color); let stand for 1 hour at 120°F. Add the sodium hydroxide to the remaining 50 g. of water, which should be very hot (180° to 200°F.); stir in the finely-ground soy protein until dispersed. Now com-

bine all ingredients and bake in a loaf pan for about 75 minutes at 375°F.; or use the canning procedure usually followed for canned meat products. Cool. This product can be eaten cold, or it can be sliced and fried before serving.

All-Vegetable "Frankfurters"

600 g. hot water	15 g. seasonings
30 g. trisodium phosphate	4 g. mixed mono- and diglycerides
255 g. granular soy protein	67 g. hydrogenated vegetable fat
30 g. 1% hydrogen peroxide	1 ml. 10% food color
27 g. hydrolyzed vegetable protein	

Combine water and trisodium phosphate, add soy protein, hydrogen peroxide, and certified food color, and mix thoroughly. Add the remaining ingredients, and again mix thoroughly. Let stand for 10 minutes. Fill frankfurter casing, link, and cook in steam at 10 p.s.i. for 10 minutes. Cool under a spray of cold water, removing casing at the same time. Frankfurters are now ready for use, or they can be stored under refrigeration for later consumption.

3. Baked and Cooked Products Based on Dough

The soy protein isolate can be added as a dry blend with sodium bicarbonate or made into a gluten-like dough according to the procedure given in Section IV. Alternatively, the neutral proteinate can be added dry or dispersed in water. Either type can be added to wheat and corn flour doughs in any desired proportion to make bread and rolls, sweet goods, biscuits, crackers, cookies, cakes, pie crust and other pastries, croutons, pretzels, doughnuts and other French-fried dough products, waffle and pancake mixes, specialty flours and mixes, dumplings, flapjacks, tortillas, and pizzas. In bread it may be necessary to increase the absorption and reduce the mixing time for best results.

High-Protein Breakfast Rolls

100 g. soy protein	14.0 g. hydrogenated vegetable fat
4 g. baking soda	3.0 g. dry yeast
200 g. warm water	0.05 g. potassium bromate
100 g. flour	3.0 g. salt
10.0 g. sucrose	

Combine soy protein, baking soda, and 180 g. of water. Knead well. Soak yeast in remaining water. Now combine all ingredients, knead, and proof for 1 hour. Punch, and shape small rolls on greased trays. Sprinkle with poppy seeds, caraway seeds, or coarse salt. Proof for 30 minutes, and bake at 350°F. for about 35 minutes. Cool.

High-Protein Crackers

180 g. soy protein	15 g. lecithin
33 g. baking soda	30 g. flour (all-purpose wheat flour)
12 g. salt	225 g. warm water
45 g. hydrogenated vegetable fat	ground caraway seeds

Combine and knead all ingredients except caraway seeds. Proof at 120°F. for 15 minutes. Roll out on floured board, and sprinkle caraway seeds on one-

half of dough. Cover with the other half of dough, and roll out very thin. Cut into cracker shapes, puncture with fork, and bake at 400°F. for about 30 minutes. Cool.

High-Protein Bread

<i>Sponge</i>	<i>Ingredients</i>	<i>Dough</i>
650 g.	patent flour	350 g.
364 g.	water	336 g.
22.5 g.	yeast	—
5 g.	yeast food	—
35 g.	shortening	—
—	50% dextrose-sucrose mix	60 g.
—	soy protein	94 g.
—	sodium carbonate	6 g.

Sponge: Mix all sponge ingredients together for 1 minute at low speed, and then for 2 minutes at medium speed. Ferment for 4 hours at 78° to 82°F.

Dough: Put water in mixing bowl and add dry ingredients. At low speed add sponge. Mix at low speed for 2 minutes, and then at medium speed for 3 minutes. Let stand for 20 minutes. Divide dough into loaves, and proof for 15 minutes. Mold dough and place in pans. Proof for 1 hour. Bake at 425°F. for about 25 minutes.

Molasses Protein Bread

250 ml. water at 120°F.	16.5 g. shortening
4 g. trisodium phosphate	75.0 g. molasses
50 g. soy protein	3.0 g. salt
5 ml. 1% hydrogen peroxide	3.5 g. dry yeast
0.3 g. potassium bromate	63 ml. warm water for yeast
400 g. all-purpose flour	

Mix the soy protein, water, and trisodium phosphate together, and stir well. Allow the mixture to stand for 30 minutes. Add the hydrogen peroxide and potassium bromate. Soak the yeast for 5 minutes in the warm water. Mix and knead all ingredients together, proof for 45 minutes, punch, and shape into loaves. Proof for an additional 40 minutes, and bake at 375°F. for 35 to 45 minutes.

High-Protein Shortbread or Muffins

200 g. soy protein	5 g. salt
10 g. sodium bicarbonate	27 g. non-fat dry milk
325 g. hot water (160° to 200°F.)	70 g. molasses
210 g. flour (all-purpose)	40 g. vegetable shortening melted
15 g. baking powder	1 egg
100 g. sugar	cinnamon, raisins, nuts, etc.

Blend soy protein and bicarbonate of soda. Sift together all other dry ingredients. In the electric mixer add the hot water to the protein-bicarbonate blend, and mix at high speed for 3 to 5 minutes. Add all remaining ingredients at once, and mix at medium speed until fairly smooth to obtain a thick, sticky dough. Cinnamon, raisins, and nuts may be added as desired. Fill *well-greased* muffin pans, and bake at 350°F. for 40 minutes, or bake in loaf pan for 1 hour at same temperature. Cool, and serve while slightly warm.

High-Protein Doughnuts

200 g. soy protein	70 g. molasses
10 g. sodium bicarbonate	15 g. baking powder
250 g. hot water	5 g. salt
150 g. sugar	25 g. non-fat dry milk
1 egg	40 g. shortening
210 g. all-purpose flour	

Mix the soy protein, bicarbonate, and water vigorously together for 5 minutes. Add sugar, egg, fat, and molasses. Mix well. Add the remaining ingredients, and work them into dough. Let dough stand for 15 minutes. Roll out, cut with doughnut cutter, and fry in deep fat at 325° to 335°F. for 3.5 minutes. Dip into sugar and serve.

All-Protein "Dumplings"

30.0 g. soy protein	2 g. salt
45.0 g. water	3 g. shortening
2.5 g. trisodium phosphate	0.4 g. monosodium glutamate

Dissolve trisodium phosphate in water, and then mix with the soy protein. Add other ingredients and knead well. Form into small balls, boil in bouillon, and serve. Alternatively add grated cheese to dough, boil in salt water, and serve with tomato sauce as a main dish; or fry in deep fat until light brown and serve as is.

4. Cereal-Type Products

Edible soy protein can be incorporated by appropriate techniques into cooked cereals; dry shredded, puffed, flaked or other breakfast cereals; baby cereals; and protein-fortified cereals.

All-Protein Cooked Cereal

450 g. boiling water	100 g. granular soy protein (farina-like grind)
7.5 g. trisodium phosphate	5 g. salt

Add the phosphate to the boiling water, stir until dissolved, and add the protein. Cook in covered double boiler for about 30 minutes with occasional stirring until desired consistency is reached. Add salt and serve.

5. Macaroni-Type Products

Although posing a somewhat difficult mechanical problem technically, macaroni products, including spaghetti, macaroni, vermicelli, ravioli, and noodles, can be fortified with soy protein by adding the latter to semolina or farina or their doughs, in any proportion desired. If the pH of the added soy protein is kept below 6, the final product will cook to a firm texture without dissolving. By proper formulation, even canned spaghetti can be firmed by use of soy protein, and farina can be upgraded in quality as an extender of semolina.

High-Protein Spaghetti (55% Protein Content)

50 g. soy protein

50 g. semolina or farina

75 g. water

1 g. sodium carbonate

Dissolve the carbonate in the water; add the soy protein, and knead until a dough is formed. The pH of this dough when dispersed in water should be between 5.5 and 6.0. Add the semolina or farina, and continue kneading. Proof the dough if desired, and knead again. Extrude through dies in the desired shapes.

6. Oriental-Type Foods

Traditional oriental-type foods are reviewed by Burnett (24), Smith (52), and others (51, 90, 91), and by Dean in Chapter 9. Dry soy protein can be used as the base to make oriental-type noodles, yuba, tofu, miso, or soy sauce. Most of these oriental foods are usually made from whole soybeans and contain the oil of the bean. Edible isolated soy protein can be dispersed at a suitable concentration and pH by means of an alkaline reagent; vegetable oil is added, the mixture homogenized, and the curd is precipitated with calcium or magnesium salts, or with acid, or with a combination of precipitating agents.

7. Specialty Foods

Soybean protein or proteinate can be added as desired to salad dressings, seasonings, gravies, stews, soups; baby, junior, and geriatric foods; dietary, convalescent, and pharmaceutical foods, survival rations, snacks, beverages, dessert specialties, and canned and frozen foods to increase protein content or to take advantage of the emulsifying, thickening, stabilizing, or other properties.

8. Confections and Preserves

Soybean protein can be added to confections and preserves as a protein fortifier (24, 92). Included are candies, marshmallows, nougats, creams, caramels, icings, frostings, meringues, jams, jellies, and chocolate products. Soy protein can be used to extend or replace egg protein in these products. Recently a patent was issued on synthetic preparation of imitation nutmeats from soy protein isolate (93).

9. Coatings

Soybean protein can be formulated into edible films and casings, and into spray, dip, or brush coatings and glazes.

Coatings for Nuts

40 g. soy protein

40 g. 5% sodium hydroxide

330 g. water

60 g. 2.5% citric acid

2 g. 1% hydrogen peroxide

10 g. glycerol

Soak soy protein in water for several minutes, heat to 140°F. with constant stirring, and add peroxide and sodium hydroxide. When completely dispersed, add the citric acid very slowly. Add glycerol. Add water to 500 g. total weight. Dip nuts into liquid dispersion, then dry in a forced-air stream. This coating will prevent leakage of oil. Two coats may be applied if desired.

10. Feed Uses

Dog, cat, and other pet foods represent a large potential market for isolated soy protein, as also do pig, chick, and calf starter and grower rations. Meat texture can be simulated by use of soy protein in canned dog food.

11. Modified Soybean Protein Products

Edible isolated soy protein can serve as an economical starting material for modification by enzymatic or acid hydrolysis (24, 30) to make whipping agents, soy sauce, and hydrolyzed vegetable protein (in this use it must compete with wheat and corn glutens). The latter is characterized by a meat-like flavor and can be used to flavor all-vegetable meat-type products, soups, gravies, and bouillon cubes.

12. Summary and Conclusions

We have given a general survey of the kinds of foods familiar in the United States and some other regions into which edible isolated soybean protein can be incorporated to advantage, either to replace or extend the more expensive animal proteins, or to improve their protein nutritional content in an economical manner. Specific procedures for manipulating the soy protein into many familiar forms have been set forth, and should serve as a basis for other food applications.

It may be concluded that edible isolated soy protein shows promise, from the standpoints of economy, nutritional value, status of isolation technology, color, flavor, and ease of manipulation into various food forms, of becoming a major vegetable protein constituent of foodstuffs for human consumption.

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CHAPTER 16

GROUNDNUTS (PEANUTS) AND GROUNDNUT MEAL

GORDON D. ROSEN

I. INTRODUCTION

The cultivated groundnut or peanut, *Arachis hypogaea* (also known as the earthnut, monkey nut, groundpea etc.), is now grown in most of the tropical and subtropical regions of the world. Wild types of *Arachis* have long been known in Brazil and have spread widely into other regions of South America during the past hundred years. The origin of the cultivated species is not known precisely; an account of its early history (1) reveals evidence of its propagation in Peru, Brazil, Mexico, and the West Indies in the late fifteenth and the sixteenth centuries. Portuguese and Spanish traders carried the plant to African coastal areas and to the East Indies soon after the year 1500. Introduction to China and India, now the leading producers, followed later, probably from the South Pacific area in the late sixteenth century. Cultivation in North America originated with plants transported from Africa during the slave trade era.

After the introduction of the groundnut as an oilseed into European markets in 1840 and its expanded cultivation as a food crop in the United States after the Civil War, large industries utilizing groundnuts developed during the present century, notably in edible oil production in Europe and Asia and in processed peanut foods in the United States. The growing demand for groundnut oil has resulted in extensive international trade, and groundnut now comprises a substantial fraction (about one-fifth) of the world supply of vegetable oils (2). Groundnut meal or cake, the main by-product of the crushing industry, makes a significant contribution to world supplies of animal feed and may well become an important article of human food in Africa and Asia. The potential of groundnut meal as a source of new industrial raw materials has been explored recently (3, 4) with substantial progress in fiber manufacture (5, 6).

Food industries utilizing peanuts call for prime, fresh kernels of

optimal organoleptic qualities. In contrast, lower grade and even damaged seed can be crushed profitably for oil and cake. With primary objectives of flavor and oil yield, respectively, food processors and seed crushers tend to regard protein quality as of only minor importance, and it is increasingly evident that more detailed and extensive studies than hitherto will be needed to ensure that groundnut products achieve their full potential as food and feed.

TABLE I
GROUNDNUT PRODUCTION IN MAJOR PRODUCING COUNTRIES^a

Country	Average annual production in the shell (1000 short tons)			
	1935-39	1945-49	1950-53	1955 ^b
India	3,296 (34) ^c	3,751 (35)	3,679 (35)	4,325
China	2,913 (30)	2,821 (26)	2,589 (25)	2,950
United States	615 (6.4)	1,046 (9.8)	831 (7.9)	774
French West Africa	876 (9.1)	812 (7.6)	837 (8.0)	1,140
Nigeria and Cameroons	355 (3.7)	582 (5.4)	641 (6.1)	1,100
World total ^d	9,590	10,715	10,488	12,945

^a General reference: U.S. Dept. Agr., "Agricultural Statistics," 1954 (see also previous years).

^b U.S. Dept. Agr., *Foreign Agr. Service Circ.* FFO 13-57 (1957).

^c The figures in parentheses express the production as percentages of the world total.

^d World production in 1956 was estimated as 13.6 million tons. See: *Food and Agr. Organization, U.N., Monthly Bull. Agr. Economics and Statistics* 6(4), 11 (1957).

Commercial, agricultural, and technical literature on groundnuts is voluminous and expanding rapidly. A United States periodical (*Peanut Journal and Nut World*) is devoted primarily to peanuts, and comprehensive bibliographies have been compiled (7-9). There has been a strikingly small number of nutritional studies in which groundnut has received more than incidental attention, despite its extensive use as food and feed. Nor has there been any detailed review of the feeding of groundnuts and groundnut products. An attempt has therefore been made to include in this chapter a comprehensive bibliography of papers dealing with nutritional aspects.

II. PRODUCTION AND TRADE

World production is approximately 14 million tons of groundnuts (in the shell) per annum, the crop ranking third in the list of oilseeds, following soybeans (23 million tons) and cottonseed (18 million tons).

Although cultivation is widespread in some forty countries (10), 83% of the average world production of 10.3 million tons for 1935–39 and 1945–53 was concentrated in five major producing areas (see Table I). Other countries producing more than 100,000 tons annually (average 1946–53) were the Argentine (125,000), Belgian Congo (166,000), Brazil (115,000), Burma (161,000), Indonesia (233,000), and Uganda (177,000) (2).

TABLE II
AVERAGE ANNUAL IMPORTS AND EXPORTS OF GROUNDNUTS (AS SHELLED EQUIVALENT)
AND GROUNDNUT OIL^a

	Groundnuts (1000 long tons)						Groundnut oil (1000 long tons)					
	1935–39		1945–49		1950–54		1935–39		1945–49		1950–54	
	Im ^b	Ex ^b	Im	Ex	Im	Ex	Im	Ex	Im	Ex	Im	Ex
China and Manchuria		126		6		86		32		1		15
French West Africa		409		176		203		6		41		74
Gambia		36		34		38						
India		855		96		38		10		21		42
Nigeria		212		208		294						13
United States				99		23	18			6		11
Canada	17		33		26		33		3		4	
France	714		188		242		1	46	10		67	
Germany	268		73 ^c		43		6				11	
United Kingdom	238		378		322		6	22	4		21	
Netherlands	167		14		18			41	2		5	
World total	1753	1801	763	742	811	829	153	184	63	81	206	206

^a General reference: U.K. Commonwealth Economic Committee, "Vegetable Oils and Oilseeds." H.M. Stationery Office, London, 1956 (see also previous years).
^b Im—imports; Ex—exports.
^c 1948–49.

Groundnut products enter international trade as whole seed, groundnut oil, and groundnut cake (meal). In 1935–39, 27% of the world crop of groundnuts was exported, of which just over one-sixteenth was traded as oil. In 1950–53, only 10% was exported and a little over one-third went as oil. The detailed disposition of seed and oil for primary exporters and importers appears in Table II.

The decrease in total groundnut exports has been due mainly to the withdrawal from the market of the major portion of Indian seed, now used domestically. Since World War II, Nigeria and French West Africa have assumed the role of leading exporters, the bulk of their supplies destined for the United Kingdom and France.

It should be pointed out that statistics on world trade in oilseed cakes and meals have been concerned usually with totals and not individual types (11). In line with the decrease in total seed exported, groundnut products have shown a decline due to a reduction in Indian and Chinese supplies. The shifting pattern of trade in groundnut cake is well illustrated by import figures (12) for the United Kingdom, the leading trader (1934-52). Before World War II she imported 200,000 to 300,000 tons per annum, 30 to 50% of her total cake imports; more than 90% came from India and the rest mainly from the Argentine. Since 1946, supply has been variable and imports have fluctuated between 5000 and 110,000 tons per annum (3 to 16% of total imported cakes), the major suppliers being the Argentine and Burma.

III. GROWTH, HARVESTING, AND CURING

Differences in variety, climate (especially rainfall), soil, cultural and harvesting practices, and pest and disease control result in striking variations in yield (400 to 1800 pounds in the shell per acre) (10) and quality of groundnuts in different regions of the world. For best results, careful timing of sowing and harvesting in relation to climate, and the aid of the expert in seed selection are essential. Two main forms of plant are distinguished—the erect (bunch) and the spreading (runner) with intermediate forms known as semispreading. In all forms the short-lived flower gives rise to the distinctive structure of the species, the peg, which penetrates the soil; its fruit then develops and matures underground. In general, bunch and spreading types are described as “early” and “late,” since they mature in 90 to 110 and 120 to 150 days, respectively, after sowing.

Ideally for groundnuts the soil should be well-drained, light-colored, loose, friable, sandy loam, well supplied with calcium and a moderate content of organic matter. Thorough ploughing, adequate spacing, use of fertilizers, weeding, hoeing, and crop rotation are important (13, 14), and vigorous combatting of insect pests and groundnut diseases at all stages is vital (15, 16). Harvesting time is best gauged by regular inspection of the stems and pods and by the suitability of the weather for curing. After wilting, the crop is field-cured and stacked on poles (4 to 6 weeks) or in windrows (2 weeks), allowing a free circulation of air to facilitate drying. Picking and cleaning of the pods is usually by hand, but these operations have been mechanized in some areas.

For further details, comprehensive accounts of the development and morphology of the seed and plant (17, 18) and of cultural practices (14, 19) may be consulted.

IV. STRUCTURE AND COMPOSITION OF GROUNDNUTS

1. Gross and Microscopic Structure

The groundnut is a dicotyledonous angiosperm, order *Rosales*, family *Leguminosae*, subfamily *Papilionatae*. Its mature pod ranges from 1.0×0.5 to 6.0×2.5 cm. and commonly bears two or three seeds. Each kernel comprises two large seed leaves (cotyledons), between which lies the germ, the whole covered by a thin coat (testa); industrially these are referred to as meats or nuts, germs or hearts, and skin or cuticle, respectively, the pod being known as shell, hull, or husk.

TABLE III
MAIN COMPONENTS OF GROUNDNUT KERNELS^a

Constituent	Content	
	Over-all range	Normal values
	%	%
Moisture	3.9-13.2	4.0-6.0
Crude protein (N \times 6.25)	21.0-36.4	25-30
Lipids	35.8-60.0	46-52
Crude fiber	1.2-4.3	2.8-3.0
Nitrogen-free extract	4.7-24.9	10-13
Ash	1.8-3.1	2.5-3.0

^a General references: J. D. Guthrie, C. L. Hoffpauir, M. F. Stansbury, and W. A. Reeves, *U.S. Dept. Agr., Bur. Agr. and Ind. Chem. AIC61*, pp. 50-78 (Rev. 1949); C. L. Hoffpauir, *J. Agr. Food Chem.* **1**, 668 (1953); M. F. Stansbury, J. D. Guthrie, and T. H. Hopper, *Oil & Soap* **21**, 239 (1944); W. D. Raymond, *Colonial Plant and Animal Products (London)* **4**, 200 (1954); W. D. Raymond J. A. Squires, and J. B. Ward, *ibid.* **4**, 206 (1954); R. G. W. Spickett, J. A. Squires, and J. B. Ward, *ibid.* **4**, 218 (1954).

The seeds, which weigh 0.2 to 2.0 g., range from almost spherical to roughly cylindrical with flattened, rounded, or pointed ends; the testa is commonly light tan or rose, light or dark red, and, less frequently, creamy white or deep purple.

The testa has a well-defined vascular system originating at the hilum. The epidermis consists of heavily cutinized protective tissue overlying layers of empty thin-walled spongy parenchymatous cells and the inner epidermis or perisperm; nuclei persist in the perisperm even after curing. Cured, undamaged testa contain no oil, starch, or aleurone grains.

Stomata occur irregularly over the surface of the cotyledons. The outer layer is cutinized, and epidermal cells contain a large central nucleus. Large spongy parenchymatous cells comprise the bulk of the seed, which is interspersed with vascular bundles branched extensively from four to six central and six to eight outer main bundles. The bulk of the cells contain nuclei, about one-fourth of the cell diameter, starch grains with a characteristic starlike hilum, and storage proteins contained in aleurone grains, in the matrix of

which are globoids. Oil occurs as a fine cytoplasmic emulsion distributed throughout the cells but staining more densely at the peripheries.

Heavy rolling of kernels distorts and shatters the cells, releasing oil droplets, but does not affect starch and aleurone grains (20). Dry roasting at 145° coagulates the aleurone grains and nuclei into a hard cen-

TABLE IV
VITAMINS OF GROUNDNUT KERNELS^a

Constituent	Content (γ /g.)	References
Biotin	0.34-1.1	27
Choline	1650-1740	28
Folic acid	2.8	
Inositol	1800	
Nicotinic acid	88-220	
Pantothenic acid	25-35	29
<i>p</i> -Aminobenzoic acid	1.6-1.7	30
Pteroylglutamic acid	0.51	31
Pyridoxine	3.0	32
Riboflavin	1.05-1.57	
Thiamine	2.5-14.0	33
Carotene + vitamin A	0.26	34
Vitamin C	58	
Vitamin E	119-530	35
α -Tocopherol	180-300	
β -Tocopherol	0	
γ -Tocopherol	150-230	
δ -Tocopherol	c. 100	
Vitamin K	Present	

^a General references: J. D. Guthrie, C. L. Hoffpauir, M. F. Stansbury, and W. A. Reeves, *U.S. Dept. Agr., Bur. Agr. and Ind. Chem. AIC61*, pp. 50-78 (Rev. 1949); C. L. Hoffpauir, *J. Agr. Food Chem.* **1**, 668 (1953).

tral mass and forms large oily droplets, difficult to express. Roasting in oil (135°) results in absorption of additional oil and the precipitation of swollen aleurone grains. Cooking in the presence of high moisture at 121° weakens the cell walls, destroys starch grains, coagulates nuclei, disperses aleurone grains throughout the cell, and forms free, easily expressed oil droplets. Live steam acts similarly and also coagulates the swollen aleurone grains. Cold-pressing (5000 to 7000 p.s.i.) fractures

most of the cells and partially crushes the cytoplasmic inclusions; pressing at 121° or over causes almost complete breakage of the latter.

2. Composition of Groundnut Kernels

Ranges and normal values for the content of the main components of groundnut kernels are given in Table III (21–26), and contents of vitamins, minerals, and carbohydrates in Tables IV (27–35), V, and VI (36–39). Proteins and other nitrogenous constituents are discussed in detail in another section.

TABLE V
MINERAL CONSTITUENTS OF GROUNDNUT KERNELS^a

Constituent	Content (mg. %)	Constituent	Content (mg. %)	Constituent	Content (mg. %)
Aluminum	100	Iodine	0.020	Silica	80
Barium	8.0–30	Iron	1.3–11	Sodium	1.0–50
Boron	2.6–50	Lead	0–50	Strontium	0.80–5.0
Calcium	20–85	Magnesium	90–340	Sulfur	190–260
Chlorine	0.50–10	Manganese	0.20–50	Titanium	30–80
Chromium	1.0–30	Molybdenum	0.80–3.0	Tin	0–5.0
Cobalt	0.030	Nickel	3.0–8.0	Vanadium	10–50
Copper	0.70–30	Phosphorus	250–660	Zinc	1.7–80
Fluorine	0.14	Potassium	540–890		

^a General references: J. D. Guthrie, C. L. Hoffpauir, M. F. Stansbury, and W. A. Reeves, *U.S. Dept. Agr., Bur. Agr. and Ind. Chem. AIC61*, pp. 50–78 (Rev. 1949); C. L. Hoffpauir, *J. Agr. Food Chem.* **1**, 668 (1953); W. D. Raymond, *Colonial Plant and Animal Products (London)* **4**, 200 (1954).

TABLE VI
CARBOHYDRATES OF GROUNDNUT KERNELS^a

Constituent	Content		References
	Over-all range %	Normal values %	
Reducing sugars	0.06–0.30	0.2	
Stachyose		Present	36
Disaccharide sugars (sucrose)	1.5–7.0	4.5	
Starch	0.9–6.7	5.6–6.7	37, 38
Pentosans	2.2–2.8	2.5	
Cellulose		2.0	39
Pectic acid-araban		4.0	39

^a General references: J. D. Guthrie, C. L. Hoffpauir, M. F. Stansbury, and W. A. Reeves, *U.S. Dept. Agr., Bur. Agr. and Ind. Chem. AIC61*, pp. 50–78 (Rev. 1949); C. L. Hoffpauir, *J. Agr. Food Chem.* **1**, 668 (1953).

It is not easy to decide which of the trace elements are true kernel constituents and which derive from foreign matter. Marked discrepancies are common in carbohydrate analyses (e.g., starch), and further characterization of groundnut polysaccharides is desirable.

The fatty acid composition of groundnut oil appears in Table VII; the physical properties of the oil have been reviewed (21, 40) and the glyceride structure partly elucidated (41, 42). Non-saponifiable constituents (22, 40) include tocopherols (0.02 to 0.06%), squalene (0.027%), other hydrocarbons,

TABLE VII
FATTY ACID COMPOSITION OF GROUNDNUT OIL FROM DIFFERENT TYPES^a

Fatty acid	Content						
	Grown in United States			Grown in Tanganyika			Commer- cial oil
	Spanish %	Runner %	Virginia %	Natal Common %	Spanish %	Valencia %	
Hexadecenoic							1.7-2.4 ^b
Oleic	43.5-49.7	61.3-64.7	52.5-57.7	40.8	39.2	39.2	60.3
Linoleic	31.9-37.0	19.9-23.9	26.4-32.4	35.9	37.2	38.2	20.3
Eicosenoic							1.3
Palmitic	8.2		6.3	9.7	8.7	10.8	8.8
Stearic	6.2		4.9	5.6	5.0	4.2	4.5
Arachidic	4.0		3.3	} 8.0	} 9.9	} 7.6	} 6.1
Behenic							
Lignoceric	3.1		2.6				
Total satu- rated	18.4-20.2	14.6-15.5	14.1-16.7				

^a General references: J. D. Guthrie, C. L. Hoffpauir, M. F. Stansbury, and W. A. Reeves, *U.S. Dept. Agr., Bur. Agr. and Ind. Chem. AIC61*, pp. 50-78 (Rev. 1949); S. P. Fore, N. J. Morris, C. H. Mack, A. F. Freeman, and W. G. Bickford, *J. Am. Oil Chemists' Soc.* **30**, 298 (1953); R. V. Crawford and T. P. Hilditch, *J. Sci. Food Agr.* **1**, 372 (1950); E. W. Eckey, "Vegetable Fats and Oils," p. 495. Reinhold, New York, 1954; T. P. Hilditch and J. P. Riley, *J. Soc. Chem. Ind. (London)* **64**, 204 (1945).

^b According to T. P. Hilditch and J. P. Riley, *J. Soc. Chem. Ind. (London)* **64**, 204 (1945).

phytosterol and other steroids (0.19 to 0.25%), methylnonylketone and methylnonylcarbinol, and terpenes. Phosphatides identified are phosphatidylcholine, -serine, and -ethanolamine (43), phosphoglyceroinositide (44) containing arabinose and galactose, and plasmalogens (45). Some 4% of C-20 to C-22 unsaturated fatty acids is found in the phosphatide (46). Organic phosphorus is also present as phytin (47, 48), and one-tenth of the phosphorus is inorganic (47).

Enzymes reported (21, 22) include catalase, glucosidase, lipolytic enzymes, phytase, glycer- and pyrophosphatases, carotene oxidase, a protease *arachain* (49), and a dehydrogenase (50).

3. Factors Affecting Quality and Composition

a. Type and Environment

Nine botanical species are recognized in the genus *Arachis*, the cultivated species *A. hypogaea* manifesting a number of different forms, *communis*, *micro-*, and *macro-carpa* (51). It is not possible to detail commercially important types herein, but classifications based on seed

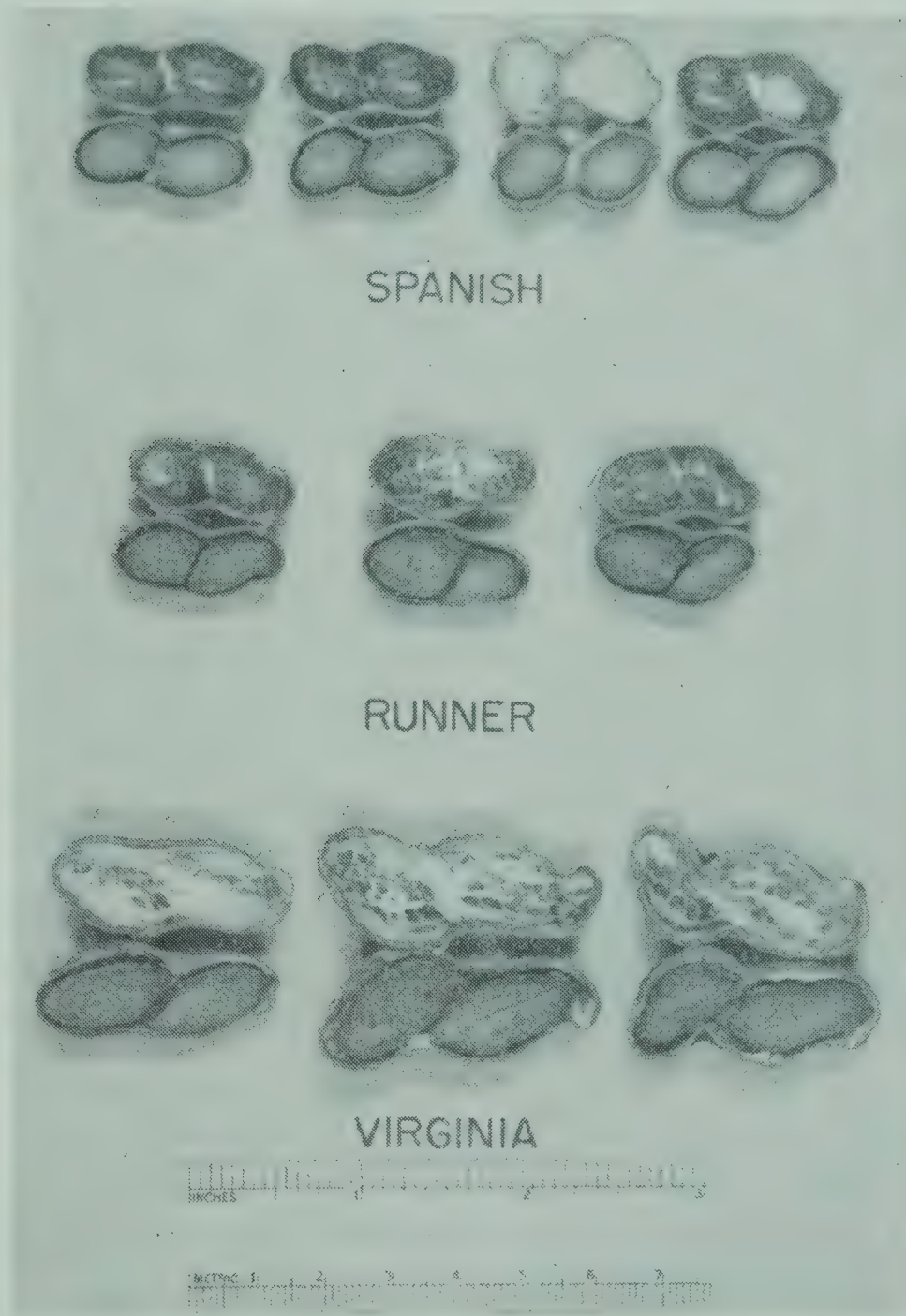


FIG. 1. Types of peanuts grown in the United States.

characteristics, such as pod and kernel weights, shape and number of kernels per pod, and testa color (18, 52) are of most value to processors. (See Fig. 1 for illustrations of some types.)

The data in Table VIII (23, 25, 26, 35, 53) demonstrate that variations in nitrogen exceed differences in oil content. It is difficult to distinguish varietal and environmental effects unless the plants are grown under identical conditions. The oil contents of some forty types so grown ranged between 46.8 and 52.0% (18). In general the large edi-

ble nuts (e.g., Virginias) have lower oil and protein contents (54, 55) and hence are not usually processed for oil and meal. For 1942, Spanish Runner, and Virginia kernels (United States) averaged 63, 61, and 58% crude protein (oil- and moisture-free), respectively (23), and identically grown types in India ranged from 47 to 57% (56). Thiamine contents of different types ranged from 2.5 to 8.1 γ /g. seed (33). For types grown in the United States, the mean contents of riboflavin and niacin were greater for Virginias than Spanish; the thiamine levels were almost equal (55). The linoleic acid content of Spanish is some 60% greater than that of Runner (Table VII), and Spanish are much

TABLE VIII
COMPOSITION OF GROUNDNUT KERNELS BY ORIGIN AND TYPE^a

Constituent	Content				
	United States Spanish	United States Runner	United States Virginia	Nigerian	Gambian
Oil (moisture-free basis) %	50.8-53.9	50.3-54.3	46.5-50.2	46.3-54.3	49.5-54.4
Nitrogen (oil- and moisture-free basis) %	7.6-11.0	8.4-10.6	6.2-10.4	7.6-9.8	6.7-11.8
Free fatty acid of oil (as oleic acid) %	0.1-3.0	0.1-2.7	0.1-1.9	0.2-10.0	0.2-10.0
Iodine number of oil	94-102	90-94	94-101	86-90	—

^a General references: M. F. Stansbury, J. D. Guthrie, and T. H. Hopper, *Oil & Soap* **21**, 239 (1944); W. D. Raymond, J. A. Squires, and J. B. Ward, *Colonial Plant and Animal Products (London)* **4**, 206 (1954); R. G. W. Spickett, J. A. Squires, and J. B. Ward, *ibid.* **4**, 218 (1954); S. P. Fore, N. J. Morris, C. H. Mack, A. F. Freeman, and W. G. Bickford, *J. Am. Oil Chemists' Soc.* **30**, 298 (1953); D. Traill and A. McLean, *Chemistry and Industry* 221 (1945).

more prone to autoxidation (57). In flavor, Spanish types are the most bland of all the raw seeds.

That environment affects composition is shown by the higher oil and protein content of Spanish seed from Texas compared with Alabama and Georgia (23). Such differences vary annually with changes in climate, soil condition, and cultural practices. Application of insecticides is a cultural practice which deserves attention in this respect. Recent studies have been concerned with a definition of safe limits needed to avoid the tainting of peanuts and derived products by means of insecticides applied during cultivation (58-61).

b. Harvesting and Curing

Various harvesting and curing practices do not appreciably affect the quality of edible seed grades (62), but in general, curing in well-

constructed stacks gives best results (58). Late harvesting results in sprouting in non-dormant varieties, and early harvesting results in a high proportion of shriveled, immature seed, which contains only 36 to 44% of oil (23, 24, 63) and is low in extractable protein (24).

c. Decortication

Groundnuts are increasingly marketed as decorticated stock; decortication is effected either by primitive manual methods or in modern plants (19) and by machines (64). Careful shelling and cleansing to yield unbroken kernels is necessary to provide groundnuts suitable for food uses and for the production of meal to be used as a source of protein fiber. Comparatively rigorous grading standards are set in the United States (65); to be graded No. 1, unshelled Spanish nuts must contain 70% of sound, mature kernels. The method of decortication influences markedly the rate of deterioration during storage. Oil mill stock received in Europe has frequently been of low quality due partly to crude methods of decortication (e.g., pestle and mortar) (24).

d. Storage

Absorption of foreign flavors is rapid at 27° but almost negligible at 1° or below; storage at both high and low moisture contents produces deleterious results. Moisture contents of 6.5% represent optimal conditions. Mold and insects can be controlled, respectively, by ozone treatment and by fumigation with a 3:1 mixture of ethylene dichloride and carbon tetrachloride (66). Only slight changes in content of oil, nitrogen, free fatty acids, and in iodine number occur in groundnuts held in closed cans and stored at 1° for up to 2 years (67). Refrigeration is recommended for bulk storage (66).

The effects of breakage, weather, molds, and insects on oil mill stock destined for Europe are sometimes accentuated by long periods of storage at tropical temperatures and humidities owing to lack of transport. Even new-crop nuts are affected in a few months, as evidenced by the oil and free fatty acid contents of wholes and halves (48% and 3%), brokenes (44% and 7.3%), and dust (22% and 69%) (24). Damage due to molds, fungi, and bacteria (16, 68) and to insect infestation (15, 69) has been described and vividly illustrated. In laboratory tests, conducted for periods of 12 months, larvae of *Trogoderma granarium* Everts caused losses in dry weight of up to 37% (70).

e. Roasting

Organoleptic and nutritional properties of groundnuts can be profoundly altered by roasting, but the chemistry of the changes involved

is incompletely understood. Moisture content decreases from a value of 4 to 6% to a value of 1% or less after roasting at internal temperatures of 130 to 150° (71). Thiamine contents are reduced from 7 to 12 γ /g. to 0.5 to 2 γ /g. when nuts are roasted at 150 to 160° for 20 to 40 minutes (72, 73). Roasting at 160° and 180° for 40 minutes causes losses of riboflavin and pantothenic acid of zero and 25, and 25 and almost 100%, respectively (74). Nicotinic acid (72) and choline (75) contents are little affected by roasting; likewise iodine value, saponification and acetyl numbers, and free fatty acid content of the oil are barely affected (76). The volatile products of roasting at 150° include carbon dioxide (98%), small amounts of aldehydes including furfural derivatives, ammonia, and volatile sulfur compounds. These are formed principally as a result of browning involving sucrose (71). Roasting at 100° for 2 hours causes a decrease in amino nitrogen (63%) which is non-specific for the free amino acids present in the raw seed. Crude fiber decreases on roasting. Roasting at 120° for 3 hours in oil reduces the water-soluble nitrogen, and in air at 150° there is a decrease in peptizable nitrogen (77). Roasting for 40 minutes at 160° does not appear to alter the amino acid composition (78, 79) but may affect amino acid availability. (For details of the effect of heat on groundnut protein see page 443, and also the general discussion on the effect of heat on proteins in Chapter 5.)

V. ECONOMICS OF USE IN FOODS AND FEEDS

In general the value of groundnuts depends on local and world supply conditions and on the availability and relative prices of alternative foodstuffs and other edible oils and fats. In the past, producers have had the benefit or borne the burden of changing conditions of supply and demand, but many governments now guarantee prices to growers. Since 1933 in the United States, there have been comprehensive annual peanut programs (80, 81) involving price supports, acreage controls, and subsidies for crushing; their effects on the value, quality, and supply of peanuts have been reviewed by Penney *et al.* (82).

Of the world crop, some 55 to 65% is crushed for oil; some of the remainder is consumed as human food, but the proportion varies considerably in different regions. Factors affecting the proportions used for food and oil include the size and quality of the crop, the price differentials between oil and food grades and between groundnuts and alternative foods, and national economic policies. Specialized cultivation and harvesting and greater care in cleansing and marketing groundnuts for commercial food applications as compared with oil mill stock are reflected in relative prices. In 1954-55, edible and oil grades in the United

Kingdom averaged £120 to 130 and £70 to 75 per ton, respectively (83); in the United States (1952), shelled No. 1 Spanish (food use only) rated 20 to 21 cents per pound compared with 16 to 18 cents per pound for No. 2 and less for lower grades (10). Size, appearance, and organoleptic qualities govern the value of edible nuts, and, as processing costs do not fluctuate much, the prices of processed foods are related closely to the cost of the nuts. Peanut products have to compete with tree nuts and spreads (80, 82).

In 1946–53, the value of crude groundnut oil in the United Kingdom (12) and the United States (10) was, respectively, 4.4 and 5.8 times that of the meal. For oil yields of about 40% it is apparent that 70 to 80% of the value of the seed lies in its oil, contrasting sharply with soybeans (only 20% oil) for which the meal is of slightly greater value. In consequence, groundnuts for crushing are traded on the basis of oil content, and processing is aimed at high oil yield, often without attention to the quality of the meal. The free fatty acid content of the oil gives a rough guide to its quality and an indication of the anticipated refining loss. Acid oil commands only 40 to 65% of crude oil prices.

On account of its higher energy value as a feedstuff, groundnut cake, containing residual oil, is sold at 7 to 8% above the price of solvent-extracted meal (83). Apart from the oil, the value of the meal is dependent on protein content. Uncorticated meal (26% protein) rates 60 to 70% of the value of decorticated meal (45% protein) (84), and, in the range of protein content of 46 to 57%, small differentials (up to 2%) obtain for the higher levels (83).

VI. WHOLE GROUNDNUTS AS FOOD AND FEED

1. Human Food

Groundnuts have been grown for centuries as a human food and are consumed both raw and in a variety of confections. Their value in human dietaries has been discussed in Chapter 9, and the following account is devoted primarily to manufacturing processes and their effects on quality. The unique edible peanut trade in the United States is a highly developed and organized industry (19, 80). Of approximately 280,000 tons used annually in 1946–50, 48% went for butter, 22% for salting, 23% for candy, and about 4% for roasting (80). By contrast, European consumption is mainly raw or roasted, with little conversion to butter. Research in this field has been largely devoted to consumer appeal with less attention to nutritive value.

The composition, digestibility, and energy values of peanut products for human subjects have been compared with those for other foods

(85). Unheated kernels are a good source of thiamine, niacin, pantothenate, and choline but are deficient in vitamins A, D, and B₁₂. The high oil content of groundnuts affords an excellent energy source.

a. Use as Nuts and in Confectionery

Raw and roasted peanuts in the shell in the United States have lost ground to salted Spanish and Virginias. The cleansed, shelled nuts are cooked in vegetable oil for 15 to 40 minutes at 140° to 160°, drained, and salted and cooled before being packed in cellophane or tins (19).

Candy and sweetmeats include chocolate-coated peanut bars containing creamy caramel, marshmallow, etc., peanuts set in sugar and syrup ("bars" and "brittle"), and sugar- or chocolate-coated peanuts. Peanuts are also used in bakery products (19).

b. Peanut Butter

The grinding of peanuts into a paste for use as food has long been practised in Spain, Rhodesia, India, etc., but commercial production on a large scale is confined to the United States where it has expanded rapidly since its origin as an invalid food late in the nineteenth century.

Peanut butter is made from single types (Spanish is preferred) or from blends (e.g., Spanish plus Virginias or Runners). High-grade seed (No. 1 shelled) is used for best quality, but cheaper grades are also marketed (19). Efficient removal of foreign matter and of discolored and shriveled kernels is essential for optimal quality. The raw nuts are roasted at temperatures ranging from 90° to 170° for 10 to 45 minutes. A light-colored roast can be produced at temperatures rising to 135° in 17 minutes, whereas heating up to 150° for 30 minutes yields a dark product (86). The roasted stock is cooled by aeration and is "blanched," after breaking on brush-plate or ribbed-rubber rollers, by blowing out the skins. The germs, which contain bitter principles, are usually screened off. Some 4% of oil-soaked skins, 2% of germ, and a further 2% of manually separated, low-grade kernels are removed. The roasted splits are then ground to a butter, and packed in sealed glass or tin containers. Additives include salt (and occasionally sugar) to flavor, hydrogenated peanut oil to reduce oil separation, vitamin A to improve nutritive value, and antioxidants to retard rancidity.

Plant and processes for peanut butter manufacture have been described by Woodruff *et al.* (87). Methods of analysis and recent research on the improvement and control of quality by attention to the specific effects of processing variables have been reviewed by Freeman *et al.* (86). Thiamine content has been proposed by Willich *et al.* as an indication of processing history (88).

Standards for peanut butter have been laid down (89). Quality is assessed largely by aroma, flavor, and appearance (color and oil sep-

aration), but spreadability, stickiness in the mouth, and resistance to development of rancidity are also important. It is used primarily as a "spread" in the home and also in confectionery.

Peanut butter contains 25% protein and, among high-protein foods, is outstanding as an energy source (about 2900 calories per pound). Provided it is not overheated during roasting it can serve as a useful source of thiamine, niacin, and pantothenic acid. Its use in admixture with yeast in the treatment of vitamin B complex deficiency has been reported (90).

c. Groundnut Milk

The local production of milk and curd substitutes from groundnuts has long been practiced in China. A patented milk substitute can be prepared from mixed extracts of groundnuts and soybeans with added fat and sugar by treatment with milk-ripening bacteria and citric acid (91).

As a contribution to the task of providing more and better food for her increasing population, a groundnut milk has been developed during the last decade in India, and earlier processes (92) have now been improved (93). Decorticated kernels are lightly roasted, deskinning, and freed of defective seeds. The kernels are milled into a paste which is stirred with five times its bulk of water, filtered, and neutralized to pH 6.6 to 6.8 with lime water. The emulsion is boiled at pH 6.8 and steamed for 45 to 60 minutes to remove its nutty flavor and odor. Only slight losses of nutrients occur during this process (94). Its nutritive value is improved by the addition of calcium phosphate (0.26%), sodium citrate (0.11%), and vitamins. Sugar or synthetic flavors can be included. The milk is finally homogenized and can then be used as such or for the production of curd, buttermilk, khova, or lactic cheese. From 1 lb. of kernels, 8 to 9 lb. of milk are produced.

Groundnut milk, thus produced, is similar in composition to cow's milk and is expected to cost only about one-third of the price of the latter. Without amino acid supplements, however, it would be inferior to cow's milk in protein quality. A pilot plant with a capacity of 500 lb. of groundnut curd per day is in operation in India (95). The next phase in this important project will involve its extension, on an economic basis, to a scale large enough to make a real contribution to the improvement of food supplies for India's millions. (See also Chapter 9.)

2. Animal Feed

a. Swine Feeding

"Hogging off" is the practice of allowing swine to feed on peanuts which they root out of the soil when turned loose in the field. Apart

from the cleaning up of waste peanuts after harvesting, a substantial acreage is planted in southeastern United States as forage for swine over 40 lb. live weight. Whether the crop is "hogged off" or not often depends on the expected cost of picking and the relative values of peanuts and hogs. During the years 1932-45 approximately 1,000,000 acres per annum were planted for "hogging" (10, 19), but the acreage has declined somewhat to 713,000 for 1946-52 (10). Dormant seeds, such as Runners, are more suitable, since swine do not relish sprouted peanuts. Cattle are frequently fed on the peanut vines, leaving the nuts for the hogs. In some areas peanuts for forage ("hogging") are interplanted with corn.

Investigations on the feeding of whole peanuts to swine have been concerned mainly with its effect on the texture of pork and ham, which tend to be softer and of lower value than non-peanut-fed types. Hams from peanut-fed hogs, though less compact and hard, have been claimed to be more juicy, more tender, and less salty than hams from corn-fed hogs (96). Though improved by alfalfa meal, mineral, and vitamin A supplements (97), peanuts are inadequate as the sole protein source for young pigs (98). For fattening stock, however, they are a valuable feed (98), provided they are fed early (99) and fattening is terminated with "hardening" corn (100) or other high-carbohydrate feed, or rations containing corn and cottonseed meal (99). Rations of corn and soybean oil meal containing 15% beef tallow were only partially successful in hardening the fat of hogs fed peanuts from 50 lb. live weight and then only if peanut feeding was discontinued at 120 lb. live weight (101). In general, not more than 100 lb. of shelled peanuts per head should be fed. Mineral supplements, particularly calcium, are essential for peanut-fed hogs (102, 103).

b. Cattle Feeding

Peanuts and whole cured plants can be fed with advantage to dairy and beef cattle (72, 104, 105), but scouring results from the use of too high a proportion in the diet. Large cattle herds raised in parts of South America are allowed to graze on perennial wild groundnut plants, some species of which seem to thrive even in hard dry soils, though others need a looser texture (1). The seeds of these forms are similar in composition and nutritive value to the cultivated groundnut (106, 107).

VII. PRODUCTION, PROPERTIES, AND USES OF GROUNDNUT MEALS

Groundnut meal (or cake) is a by-product of groundnut oil production. Much of it is used for animal feeding as such or in com-

pounded feeds. Though it has long been recognized as a valuable feeding stuff, less is known of its composition and nutritive properties than about soybean and cottonseed meals. Substantial quantities go as fertilizer (as a source of nitrogen, phosphate, and potash), especially when the price is low or the meal is very dark (19).

Cake produced in primitive presses in Asia and Africa is consumed as food, but there is no commercial production of groundnut flour for human consumption at present. In contrast to soybean and cottonseed, groundnut products do not appear to have been used commercially as components of nutrient media for antibiotic production (108, 109). Groundnut meal as a source of protein isolate is referred to in other sections and in Chapter 10.

1. Processing Methods

Groundnuts are crushed industrially for oil and cake by (1) hydraulic pressing, (2) continuous horizontal screw-pressing (expelling), or (3) prepress solvent extraction.

Mechanical expression alone (hydraulic or screw) leaves some 5 to 10% residual oil in the cake; solvent extraction is necessary for higher recoveries. The choice of process and the plant design depend on the throughput required, the relative availability and cost of skilled and unskilled labor and of solvents, and the need for flexibility to allow processing of other oilseeds. Detailed descriptions of oil mill equipment have been published (110–114), and the processes are described in Chapter 4.

Decortication of groundnuts takes place at the source or in the oil mill. (Crushing of the undecorticated stock yields low-grade oil and cake of high fiber content and is now practiced only on a restricted scale.) Large stones and trash are screened out, and iron debris is removed by magnetic separators. Oil quality can be improved by screening out powdered debris.

The cleansed seed is kibbled by passage through a breaker roll and is then cooked (to coagulate the protein, to free the oil, and reduce its viscosity), usually in a vertical, four- or five-compartment steel cooker-dryer (kettle) by means of jacket or live steam. It is not easy to specify the time-temperature-moisture relationships obtaining in the cooker, since they vary from mill to mill (19, 110, 111, 115–118), ranging from 20 to 120 minutes with temperatures rising from 80° at the top to 95° to 115° or even higher at the base. Heavy rolling before cooking (e.g., Anglo-American) tends to yield too soft a charge for pressing. Cold-pressed edible (virgin) oil is produced from uncooked seed.

In the hydraulic press process, the cooked seed is molded into cakes.

wrapped in hair cloths, and inserted into the compartments of the press. Pressures of 4000 to 5000 p.s.i. or more reduce the oil content of the cake to 5 to 10%. Oily edges are pared off the cake and returned to the incoming feed. Anglo-American presses are commonly used, but cake types are sometimes preferred (110).

Alternatively the oil is expressed from the cooked meats (at about 4 to 6% H_2O) in screw presses consisting of a tapered barrel carrying a heavy revolving screw which subjects the charge to increasing pressure as it is forced through. High-pressure screw presses can reduce the oil content to about 7% in a single-stage operation, but increased throughput is achieved by two- or three-stage operations, with rerolling and cooking between stages. A low-pressure, high-capacity machine is used for the first stage. Residual oil contents for a typical three-stage process are 26, 16, and 6%, respectively. A more granular condition for the input to the first stage is commonly achieved by recycling a small proportion of expelled cake.

In the prepress, solvent-extraction process, single- or double-expelled cake (15 to 25% oil) is solvent-extracted in batch or continuous operation (110, 112) with hexane or trichlorethylene as the solvent. Crude oil extracted with the latter is darker in color but contains more phosphatide, a useful by-product. Solvent is removed from the meal by means of jacket and live steam in various types of desolventizers (110, 112, 113). Temperatures during solvent removal are held relatively low until the final stages when, in efforts to ensure complete recovery of solvent, the meal temperature may well exceed 100°.

Pilot-plant investigations on batch (119) and continuous (120) systems of direct solvent extraction of groundnuts have been reported. The novel filtration-extraction process (121), involving extraction in a rotary vacuum filter, has as yet only been applied to groundnuts on a laboratory scale (122). An important feature of these studies lies in attempts to form a crisp, incompressible charge which is free of troublesome fines and drains well. Partial success has been achieved by preheating, wetting before cooking and drying (122), and meal recycling (123). The results of these exploratory studies may encourage seed crushers to consider further the feasibility of the commercial development of direct extraction.

Specialized mills have been developed for the production of groundnut meal as a source of protein isolate and of flour for use as human food. For both uses, the removal of skins or skin pigment is necessary (124–127). Production of solvent-extracted meal for isolate manufacture (128) involves removal of fine debris, small splits, and testa, and low-temperature vacuum desolventization. Food-grade screw-

pressed flour (129) is prepared by washing and drying the seed, removing the skins and germs, and processing at low temperatures. The cake is then ground to a very fine flour in a microcyclomat or equivalent equipment and gently roasted at 80° to 120°, to improve its flavor.

2. Composition of Meal

Data on the influence of variety, storage of seed and meal, and processing variables on the composition of groundnut meal are scanty,

TABLE IX
MAIN COMPONENTS OF GROUNDNUT MEALS^a

Type of meal	Composition					
	Dry matter %	Crude protein (N × 6.25) %	Oil %	Crude ^b fiber %	Nitro- gen-free extrac- tives %	Ash %
Groundnut cake or cake meal (decorticated) (screw- or hydraulic-pressed)	88-94	39-51	5.0-10	4.6-7.0	18-27	4.0-6.0
Groundnut cake or cake meal (undecorticated) (screw- or hydraulic-pressed)	89-93	30-35	9.0-10	22-24	21-22	5.0-6.0
Extracted groundnut meal (decorticated)	88-94	49-57	0.6-3.0	4.0-5.7	22-29	4.9-6.0
Extracted groundnut meal (undecorticated)	92	32	1.9	25	29	4.3
Groundnut flour (extracted or pressed)	92-96	50-66	0.5-10	2.0-3.2	21	3.6-4.6

^a General references: N. J. Morris and F. G. Dollear, *U.S. Dept. Agr., Bur. Agr. and Ind. Chem. AIC 151* (1949); H. E. Woodman, *Ministry of Agr. & Fisheries Bull. 48*. H.M. Stationery Office, London (1954); F. B. Morrison, "Feeds and Feeding," 22nd ed. Morrison, Ithaca, New York, 1956; B. H. Schneider, "Feeds of the World." West Va. Agr. Expt. Sta., Morgantown, 1947.

^b A product containing about 14% fiber is sometimes referred to as "semidecorticated."

though a little can be inferred from the characteristics of the whole seed. Differences in composition of commercial meals, which range in color, from pale, almost white to dark chocolate brown, would seem to merit further study.

a. Main Components

The composition of various products is given in Table IX (8, 130-132) and is dependent on the extent of decortication and the method

of production. Fine ground shell is used in the United States to adjust the crude protein to trading rule levels, usually 45% (19).

b. Proteins and Other Nitrogenous Constituents

The nitrogen content of oil-free, moisture-free groundnut meal is close to 10%. Since purified groundnut proteins contain 18.3% nitrogen, the corresponding conversion factor (5.46) indicates a crude protein content of 55% (133). For present purposes, however, the conventional factor of 6.25, used for most feeding stuffs, is retained.

The proteins of undenatured meal, classified as "globulins," are readily soluble in saline (5 to 10%) and in neutral, acid, and alkaline media except in the pH range of 3.0 to 5.5 and below pH 1 (47, 116, 117, 134, 135). Storage of nuts and meal at 24° or below does not greatly affect their nitrogen solubility in water (136) or saline (137). Fractionation of the saline-soluble protein by precipitation with ammonium sulfate yields 19 parts of arachin (40% saturation), 3 parts of conarachin I (65%), and 4 parts of conarachin II (85%) (138, 139). The physical and chemical properties of groundnut protein and its fractions have been reviewed by Arthur (3). For details of protein isolation, see Chapters 3 and 10.

The "true" (copper-insoluble) protein content of the meal is 89 to 95% (137, 140), and non-protein nitrogen, as nitrogen soluble in trichloroacetic acid, ranges from 5.7 to 11%, depending on the concentration of the acid and the temperature of the solution (117, 134, 141). Non-protein nitrogenous constituents include 1.8% of purine bases (142), of which xanthine, guanine, and adenine have been isolated (143); small amounts of a number of free amino acids (71), sarcosine (144), glutathione (143), and proteose (143) are present.

The amino acid composition of groundnut meal and proteins is given in Table X (145–156). Glutamic and aspartic acids and arginine total almost 50% of the protein. Of the ten essential amino acids, arginine and histidine alone are as abundant as in whole egg protein. Its low content of methionine makes arachin an attractive protein for nutritional studies on methionine requirements. The contents of six amino acids in Indian, Spanish, and Virginia groundnuts (157) were similar, but more recent assay methods have yet to be applied to studies on differences between types.

c. Vitamins

It is difficult to calculate the vitamin content of meal from the data of Table IV because of possible removal with the oil or destruction

TABLE X
AMINO ACID COMPOSITION OF GROUNDNUT MEAL AND PROTEINS^a
(Expressed as grams per 16 g. total N)

Amino acid	Groundnut meal		Groundnut protein isolate		Average composition for meal and isolate	Whole egg protein	Per cent surplus or deficit of groundnut protein relative to whole egg	Arachin M	Con-arachin M
	C ^b	M ^b	C	M					
Arginine	10.4	11.6	13.1	13.3	11.8	6.6	+79	11.0	14.5
Histidine	2.9	2.4	2.3	1.7	2.4	2.4	0	2.1	1.8
Isoleucine	3.3	4.2		3.9	4.0	7.7	-48	5.0	3.5
Leucine	6.4	6.5	6.5	6.7	6.5	9.2	-29	6.5	5.8
Lysine	3.3	3.3	3.3	3.1	3.3	7.0	-53	2.0	4.1
Methionine	0.8	0.9	1.2	1.0	0.9	4.0	-77	0.6	1.9
Phenylalanine	5.1	5.2	4.7	5.3	5.2	6.3	-17	5.6	3.8
Threonine	2.6	2.8	2.9	3.1	2.8	4.3	-35	2.5	1.7
Tryptophan	0.9	1.1	1.2	0.9	1.0	1.5	-33	0.7	1.1
Valine	3.7	4.9	3.3	4.6	4.5	7.2	-37	3.9	3.2
Methionine + cystine	2.0	2.3	2.8	2.6	2.3	6.4	-64	1.6	4.7
Alanine	4.0		3.0		3.7				
Aspartic acid	11.4	13.0		12.0	12.4	2.4		12.9	2.8
Cystine	1.2	1.4	1.6	1.7	1.4			1.0	
Glutamic acid	18.6	18.3		20.0	18.9			19.9	
Glycine	6.1	5.4		4.1	5.4			2.9	
Proline	3.5	5.0	5.3		4.4			5.5	
Serine	4.8		6.4		5.3			9.1	
Tyrosine	4.1	2.9	6.0	4.4	4.3			4.1	
"Ammonia"	2.0		2.2		2.1				
References		^c 78, 148-152	153	152, 154, 155		145		152, 155, 156	152, 155

^a Detailed references to older chemical and microbiological assays have been compiled by R. J. Block and D. Bolling, "The Amino Acid Composition of Proteins and Foods," 2nd ed. Charles C Thomas, Springfield, Ill., 1951.

^b C, chromatographic methods; M, microbiological assay.
^c Private communication from F. J. Ley and from E. J. Bigwood and G. M. Ellinger; methods used are given in references 146 and 147.

during processing. The wide range in thiamine contents [Table XI (158–171)] of various meals is due largely to this vitamin's thermal instability. Groundnut meal is notably deficient in vitamins A, D, and B₁₂ but contains high levels of choline and niacin. It has recently been demonstrated* that a sample of groundnut meal contained larger amounts (0.00238 γ /g.) of vitamin B₁₂, as measured by *Ochromonas malhamensis*, than would be expected from traces found in undamaged kernels. It is suggested that the vitamin B₁₂ may result from fermentation processes during storage of the seed prior to processing.

d. Miscellaneous Constituents

Of the mineral elements (Table XI), calcium is present at low levels, and much of the phosphorus occurs in phytic acid, phytates, and phospholipids. Approximately one-half of the seed phosphorus is removed by pressing and extraction (172). The proteins of high- and low-quality groundnuts contain 1.35 and only 1.09% of total sulfur, respectively (115); arachin and conarachin contain 0.4 to 0.5% and 1.1 to 1.2%, respectively, and the so-called minor proteins may contain even more (about 2.9%) (141). In meal from poorly cleansed seed, the insoluble ash may be over 1%.

An estimate of meal carbohydrates can be obtained from Table VI by allowing for oil removal. Citric (0.16%) and malic (0.27%) acids are present (21), and oxalic acid has been isolated (143). Testa pigments and their condensation and degradation products are found in meal. A trypsin inhibitor is present in groundnuts. The urease activity of undenatured meal is only one-hundredth that of unheated soybean meal.† Reactions claimed to distinguish groundnut from other oilseed meals have been described (173).

3. Factors Affecting Nutritional Value of Groundnut Meals

Factors affecting the nutritional value of groundnut meals, revealed by chemical and microbiological assay and nutritional studies on mice, rats, and dogs, are discussed herein. Nutritional studies and practical feeding trials on farm animals follow in another section.

a. Nutritive Value of Groundnut Protein for Laboratory Animals

Compared with whole egg protein, groundnut protein is particularly deficient in methionine, lysine, and isoleucine (see Table X).

Digestion coefficients for rats range from 82 to 98% (165, 174–178), and individual amino acid availabilities range from 95 to 100% (150). Even *in vitro* as much as 82% hydrolysis has been achieved by successive pepsin and pancreatin treatments (179). Protein efficiency ratios for the growing rat of 0.87 to 1.95 g. of gain per gram of protein consumed have been reported (165,

* J. E. Ford and M. C. Holdsworth, private communication.

† G. D. Rosen, unpublished data.

TABLE XI
VITAMIN AND MINERAL CONTENTS OF GROUNDNUT MEALS

Vitamins	Content (γ /g.)	References	Minerals ^a	Content (%)	References
Carotene	0.18	158	Calcium	0.07-0.20 (0.12) ^b	130-132, 160-162, 167-170
Choline	1760-2440 (2078) ^b	28, 158-160	Chlorine	0.014-0.030 (0.02)	130, 171
Nicotinic acid	171-250 (196)	158, 160-164	Iron	0.003-0.010 (0.007)	162, 167, 170
Pantothenic acid	15-53 (36)	158, 160, 161, 165, 166	Magnesium	0.22-0.51 (0.30)	161, 162, 169, 170
Pyridoxine	6.4	161	Manganese	0.0018-0.0055 (0.0039)	161, 169, 170
Riboflavin	2.0-5.3 (3.2)	158, 160-164	Phosphorus	0.35-0.57 (0.53)	130, 131, 160-162, 167-170
Thiamine	1.6-17.2 (8.2)	32, 72, 158, 160-165	Potassium	1.1-1.2	131, 168-170
Cobalamin	0.0007	^c	Sodium	0.005-0.07	170, 171
			Insoluble ash	0.1-3.0 (variable)	

^a Other trace elements present (170) (in parts per million) include barium 9.9, chromium 3.1, cobalt 0.29, copper 18, lead 0.90, molybdenum 7.8, nickel 5.1, silver <0.25, strontium 13, tin 0.80, titanium 78, vanadium 3.0, zinc 20.

^b Data in parentheses are average values.

^c W. F. J. Cuthbertson and F. B. Brown, private communication.

180–186), averaging 1.5 g. for a level of 8 to 10% protein in the diet fed for 6 to 10 weeks. Three reports (182, 184, 186) demonstrate lower efficiency at high levels of intake. Net protein utilizations at 8 to 10% in the diet (165, 174, 175, 177, 178, 187–189) range from 38 to 65 with a mean of 50; at 22% in the diet the net protein utilization was only 26 (178).

The nutritive value of groundnut protein has also been assessed on adult rats (151, 177, 190, 191), mice (151), and dogs (151, 192, 193) by means of growth, nitrogen metabolism, and repletion techniques. Biological values of 46 (adult rat) and 61 (dog) and protein efficiencies of 64 (mouse) and 58 (dog), relative to casein at 100, were obtained. In a microbiological assay of the quality of intact proteins (194), groundnut gave a relative nutritive value of 52 (casein = 100).

Methionine is the limiting amino acid for growing rats fed groundnut as sole protein (178, 186, 195), additions of 0.4 to 0.5% DL-methionine effecting a marked improvement. Cystine exerts a sparing effect (178), and threonine (186) and lysine (195) improve further the methionine-fortified protein. Failure in some experiments to increase growth (196) or biological value (197) with methionine may have been due to the use of rats of too high an initial weight.

At a level of 8% in the diet very little growth in mice occurs on arachin (156). At a level of 20%, rats fed arachin grew at only one-seventh of the rate achieved on whole groundnut protein or conarachin (198). Methionine addition (0.4 to 0.5%) trebled the growth rate on arachin (198, 199); homocystine is said to stimulate growth (200), but not so for cystine (199, 200). Tryptophan has been reported as a secondary deficiency (198, 201).

Reproductive performance on groundnut as sole source of protein is poor, as judged by stillbirth, viability, and lactation (154) in mice bred on diets containing isolated protein at the 19% level. Methionine and lysine addition improved performance slightly. For rats, methionine, but not cystine, improved growth and lactation (202).

In admixture with ordinary and enriched wheat flour (161, 182, 203–206), groundnut meal improves growth and protein efficiency by some 20 to 50%, with few exceptions. Diets containing corn proteins are likewise improved by supplements of groundnut meal (180, 204, 205, 207, 208). Beneficial effects of admixture of groundnut meal with lucerne (209), oatmeal (209), and sunflower cake (210) have been reported.

Many of the aforementioned tests included comparisons with other proteins. In general, groundnut protein is superior to that of wheat but inferior, as sole or supplementary protein, to cottonseed and soybean protein of optimal nutritive value; higher levels of groundnut must be fed to equal their performance.

b. Toxins, Inhibitors, and Non-Protein Constituents

An alkaloid, arachine, which causes temporary paralysis in frogs and rabbits, has been reported present in groundnut meal (211). Toxicity attributed to groundnut meal (21) has often been traced by microscopy (212) to contamination with small amounts of castor meal or beans containing ricin, which, after concentration, is detectable by an agglutination test (213).

Fed at excessive levels, groundnut meal proves toxic for both rats (214) and dogs (215). In dogs it can cause a lethal fatty hepatitis (215), but when fed at 30% with bread and horsemeat it leads only to serum disturbances and slight modification of hepatic mitochondria (216).

A trypsin inhibitor is present in groundnut meal, manifesting approximately half the activity of the well-known soybean inhibitor (217). Concentration (115) and isolation (195) of the groundnut inhibitor have been reported, but details have not yet appeared. When added to screw-pressed meal, it suppressed the growth of rats (195).

Although it is a concentrated source of protein and may contain useful quantities of choline, thiamine, and pantothenic and nicotinic acids, groundnut meal is a poorly balanced foodstuff. It is markedly deficient in energy-yielding fats and carbohydrates, calcium, sodium chloride, vitamins A and D, and some of the B group; much of its phosphorus occurs as phytic acid. These imbalances must be corrected to ensure satisfactory growth in rats (218–221). Solvent-extracted, but not pressed, groundnut meal is deficient in lipid factors capable of averting sterility in female rats (222) and retarded testes development and degeneration of the spermatic cord in males (223) fed semi-purified casein diets. Vitamin B₁₂ and aureomycin did not stimulate rat growth on unheated or screw-pressed meal (195).

c. Effect of Heat

Roasting peanuts at 204° to 232° for 30 to 35 minutes is said to cause only a slight reduction in digestibility and biological value of the protein for rats (174). In contrast, in another report, a sharp decrease in growth was observed when rats were fed peanuts roasted for 40 minutes at 180°, compared with those heated at 160° (224). Lysine supplementation improved growth on the heat-damaged nuts. Heating at 150° for over 45 minutes and autoclaving at 120° for 60 minutes have been reported as deleterious, but boiling in water for 40 to 60 minutes did not impair nutritive value (224, 225). Further trials are needed to clarify these conflicting observations.

Mitchell *et al.* (165) found net protein utilizations for rats of 58 and 47 to 55, respectively, for low-temperature (75° to 80°) ethylene dichloride-extracted meal and screw-pressed cake (cooked at up to 119°). Net protein utilizations, calculated from the data of Cama and

Morton (178) on rats, are 40, 42, 40, and 38, respectively, for low-temperature hexane-extracted meal, moderately heated, thrice-expelled cake, and lightly and severely steamed, solvent-extracted meals; these had salt-peptizable nitrogen contents of 82 to 88%, 71 to 74%, 70 to 73%, and 26 to 31% (115). Judging over-all on growth, protein efficiency, and nitrogen metabolism, Cama and Morton concluded that mild heating was beneficial, presumably owing to destruction of the trypsin inhibitor, but severe steaming was deleterious. Thermal inactivation of the antitrypsin has been confirmed (195); the inhibitor is apparently not affected by heating at 121° for 30 minutes (226). Gross overheating reduces the nutritive value of groundnut meal for the protozoan *T. pyriformis* (194).

Peptization characteristics of oilseed meal nitrogen have been proposed as criteria for assessment of the quality of meals as raw material for protein isolate production and also as indices of nutritive value. The water, salt, and alkali solubilities of groundnut protein are reduced by heat. Meal or nuts subjected to dry heat are appreciably denatured only above 118°, but, at high humidities, temperatures greater than 80° cause insolubilization (117). The salt-peptizable nitrogen of hydraulic-pressed meals ranged from 35 to 86%. Light cooking and screw-pressing had only a small effect, but heavy steaming during devolventization not only reduced the salt-peptizable nitrogen from 70 to 30%, but also the sulfur content of the protein from 0.94 to 0.77% (115). Dry heat at 100° does not cause sulfur loss. It should be noted that the methionine plus cystine sulfur of groundnut protein accounts for only 0.59%, and the nature of the remainder has yet to be discovered. Further studies involving animal feeding are needed to assess the value of peptization characteristics for the prediction of the nutritive value of groundnut protein. (See also discussion in Chapter 17.)

The effect of heat on the vitamins of whole groundnuts has been discussed previously. No correlation between the nutritive value and the thiamine or pantothenic acid content of the meal was found in rat-feeding experiments (165).

Further research on the effects and interactions of time, temperature, moisture, and oil content on composition and nutritive value is needed to obtain a clearer picture than is at present available of the nature of the changes undergone by groundnut protein and its associated trypsin inhibitor during processing.

4. Use in Poultry and Livestock Feeds

Its high protein and low fiber contents make decorticated groundnut cake or meal a valuable supplementary protein concentrate for all

classes of farm animals. Undecorticated cake, however, contains over 20% fiber and is fed only to ruminants. In compounded animal feeds much groundnut meal is used in admixture with other oilseed meals and animal protein concentrates. In regions where groundnuts are crushed, the cake is used locally as an economic feeding stuff for livestock on the range and is supplied in the form of kibbled cake (nuts) or as cubes or pellets made from meal.

Conflicting reports on the palatability of groundnut products are due to differences in the quality of the parent seed, in processing methods, and in storage conditions; sharp, acrid products should be avoided. In contrast to soybean, there have been no reports of toxicity due to trichlorethylene-extracted groundnut meal.

Digestion coefficients and other nutritional data on groundnut products are summarized in Table XII (130-132, 158, 227, 228). These afford a rough basis for the rationing of livestock, but strictly they are applicable only to the experimental circumstances of their determination. In formulating rations, allowance is made for the mineral and vitamin deficiencies of groundnut meal (see Table XI). For breeding stock, all-vegetable rations containing groundnut meal are inadequate, and provision of animal protein and associated factors is essential.

a. Poultry

In the light of its amino acid composition (see Table X) and recommended allowances (229) for young chicks, groundnut meal can be expected to be deficient primarily in methionine plus cystine and inadequate also in lysine and tryptophan. In fact, on rations containing 20% groundnut as sole source of protein, the average daily gain can be increased from 3.3 to 5.8% by supplementation with methionine alone and further to 6.9% by addition of methionine plus lysine (230). For cereal-groundnut combinations, however, lysine can become the limiting factor.

Growth of young birds on milo-groundnut rations, fortified with vitamin B₁₂, was good when methionine and lysine were added, but methionine alone was ineffective (231). Groundnut protein (3.3% lysine) has been found inferior to soybean [5.4% lysine (145)] as a cereal supplement (232, 233), and this is confirmed by gross protein values of 33 to 54 and 57 to 85, respectively (casein = 100) (234). The reported equivalence of groundnut and soybean meals (235, 236) may well have been due to the feeding of excess protein. Thus Marvel *et al.* (235) fed 8% corn plus 15% oilseed proteins; and it has been demonstrated that the high growth rate on 8% cereal plus 8% fish meal proteins can be equaled by feeding 8% cereal plus 16% groundnut (237). It has been suggested that poultry requirements for vitamin B₁₂ are lower in all-plant rations in which groundnut replaces soybean meal (238,

TABLE XII
NUTRITIONAL DATA ON GROUNDNUT PRODUCTS FOR DIFFERENT ANIMAL SPECIES

Material	Animal species	Digestion coefficients					Nutritive ^a ratio	Total digestible nutrients (%)	Starch equivalent (lb./100 lb.)	References
		Crude protein (%)	Fat (%)	Crude fiber (%)	Nitrogen-free extractives (%)					
Decorticated groundnut cake and meal	Cattle	90	91	32	83		1:0.8	77	—	132
	Sheep and Goats	91	91	8 ^e	85-90		1:1.1	82	73	130, 132
	Swine	94	56 ^b	56 ^f	85		1:0.9	73-82	—	132, 140
	Poultry	82-94	81	7	81				67-88 ^d	227, 228
Undecorticated cake and meal	Sheep	92	79 ^b	12	69 ^b		1:1.0	—	44 ^b	130
	Poultry	70	90 ^c		85 ^c				57 ^c	227

^a See F. B. Morrison, "Feeds and Feeding," 22nd ed., p. 41. Morrison. Ithaca, New York, 1956.

^b Extracted (low oil content).

^c Pressed.

^d Productive energy (therms per 100 lb.).

^e H. F. Woodman, *Ministry of Agr. & Fisheries Bull.* 48. H.M. Stationery Office, London (1954).

^f B. H. Schneider, "Feeds of the World." West Va. Agr. Expt. Sta., Morgantown (1947).

239). The nature of the factor in alcohol-washed groundnut meal, found to stimulate chick growth on partly-purified casein diets (240), has not yet been elucidated.

The influence of processing on the quality of groundnut meal for poultry has only been superficially studied. In semipurified rations containing meals heated to various extents, there appeared to be no difference in lysine availability (241). Meal from red- and white-skinned varieties and protein isolated from the former were equivalent, and low-temperature solvent-extracted meal was equal to commercial hydraulic cake heated to 107° during cooking (236). The use in these experiments (236) of rations containing 20% proteins, of which only one-quarter was groundnut and which contained animal sources also; may have obscured real differences in these meals. Gross protein values of lightly and heavily steamed solvent meals [types D and C (115)] were 52 and 42, respectively,* demonstrating for young poultry the deleterious effect of overheating. The nitrogen of these meals is soluble in alkali (0.02 N NaOH) to the extent of 91 and 55%, respectively.†

Groundnut meal can effectively replace a portion of soybean meal (232, 242) in practical poultry rations. Though groundnuts (243) and groundnut meal have been used (244) for turkeys, there are few reports on performance. As a partial replacement of meat scraps, groundnut meal significantly reduced mortality (245).

For intensively housed birds, all-plant rations fed ad libitum equaled rations containing animal by-products in growth promotion and feed conversion at the point of lay (21 to 24 weeks), despite a definite inferiority in the first 6 weeks (246). Starting (18% protein) and growing (16%) rations contained approximately equal parts of cereal and groundnut protein. This transitory inferiority was eliminated by doubling the amount of groundnut meal or by the inclusion of small amounts of fish meal or solubles. Aureomycin (0.002%) in these rations stimulated growth throughout the 21 weeks but not feed conversion efficiency. The all-plant starter rations were supplemented with riboflavin to ensure a level of 0.3 mg. per 100 g. of feed. It is doubtful whether such satisfactory results would be achieved at lower overall protein levels obtaining, for example, if the rations were fed in combination with scratch grain.

Laying birds fed corn and peanuts ad libitum gave poor egg production, and body and egg fat were soft (247). The replacement of fish or meat protein in laying rations with groundnut meal has been attended by both satisfactory (248–250) and unfavorable (251–253) results. For egg production, methionine rather than lysine appears to be the limiting amino acid. The methionine and methionine-plus-cystine requirements for egg production have been reported as 0.38% and 0.63% of the ration, as determined on rations containing 37% groundnut meal (253).

Poultry fed on all-plant rations from hatching to eighteen months were equal in laying performance to those that had received animal by-products or aureomycin (254). Thus, laying rations (all mash) containing 14.5%

* J. Duckworth and G. N. Ellinger, private communication.

† G. D. Rosen, unpublished data.

protein (10% cereal, 4.5% groundnut) supported egg production and food conversion over 52 weeks equal to those on rations containing 2.5 to 7.5% fish meal or higher levels of groundnut meal (8.5% protein). At more critical protein levels (8% cereal + 3% supplement), groundnut meal was significantly inferior to fish meal in egg production (down 30 to 40%), feed conversion efficiency, and body weight gains. Limited access to unfermented droppings (daily cleaning) during rearing and laying appeared adequate to sustain egg production. Birds on wire (in laying pens), however, laid fewer eggs during the last 6 months. An important observation on housing was that, on laying rations containing 11% of protein (3% groundnut), egg production in straw yards was lower than in laying pens. Respective protein conversion efficiencies were 15.4 and 17.3%. With an additional 3% barley protein in the ration (14% protein) no such difference was observed.

b. Swine

The use of whole peanuts as forage for swine has been described previously. In the light of estimated requirements (255), groundnut meal is deficient in lysine (40%) and methionine plus cystine (25%) as sole source of protein for young pigs. In combination with cereals (low lysine content) the lysine deficiency is accentuated (*vide infra*). The deficiency of methionine is illustrated by the inability of groundnut meal, as against sunflower or linseed meal, to supplement a palm kernel meal-cereal-fish meal ration (256). Mineral and vitamin deficiencies must be eliminated if swine are to thrive on feeds containing large quantities of groundnut meal (103, 257). In order to avoid soft body fat (see also Section VI), the feeding of 6 parts of milo chops (258) or 5 parts of corn meal (259) for each part of groundnut press cake has been recommended.

Brief trials on vitamin and antibiotic supplementation of yellow corn-groundnut meal rations for piglets from 20 lb. live weight have been reported. Stimulation in rations containing 12 to 23% protein by an animal protein factor (A.P.F.) concentrate (260-263) was greater for groundnut than for soybean and fish meal rations (264), but vitamin B₁₂ (tested with 23% protein in the diet) was ineffective (260, 261). Methionine did not enhance the response to A.P.F. (261). Aureomycin (263, 264) stimulated growth (23% protein), but penicillin did not (265). Streptomycin (265) and vitamin B₁₂ (266) evoked small but definite responses. For fattening pigs (over 100 lb.) reduction of aureomycin to a level of 10 g./ton did not affect live weight increases, but complete withdrawal retarded growth significantly (267).

In practical feeding trials (268, 269) groundnut meal was equivalent to fish meal as a supplementary protein source for the range of 50 to over 200 lb. live weight. Daily gains of 1.83 and 1.53 lb. on groundnut meal-tankage (9:1) and tankage, respectively, as supplements to corn have been observed (257). Other fattening trials (131, 270-274) have revealed satisfactory gains and economy on groundnut meal, but conflicting results on its value relative

to fish meal, soybean meal, etc. Though of considerable practical importance, the results of these trials do not afford a valid comparison of protein qualities for swine because excess protein was involved in many cases and the trials were in effect a comparison of productive energy content.

A set of conditions for the comparison of the quality of concentrates as sources of supplementary proteins for growing pigs (40 to 100 lb.) has been described (275). On a basal ration of barley and wheat feed (2:1) with added grass meal, vitamins, and minerals, supplements of white fish meal (7%) and groundnut meal (8%) supported gains of 0.92 and 0.73 lb./day, feed conversion efficiencies of 3.1 and 3.9, and nitrogen retentions of 42 and 36%, respectively. To equal the performance on fish meal, 20% of groundnut meal [cf. 15% soybean meal (276)] had to be included, thereby raising the total crude protein from 14.2 to 18.5%. With a mixture of 6% groundnut meal and 2% fish meal, a growth rate 95% of that on the standard fish meal (7%) ration was achieved. For rations containing 20% of groundnut meal, in the period between 35 and 80 lb. live weight, it has recently been found* that the addition of 0.2% DL-methionine plus 0.2% L-lysine monohydrochloride improved significantly the mean daily gain from 0.86 to 0.98 lb. and the feed conversion efficiency from 3.06 to 2.70 lb. per lb. of live weight gain.

The addition of 3% condensed fish solubles to a barley-wheat bran-lucerne meal basal ration, supplemented with 8% groundnut meal (total crude protein = 15.7%), failed to achieve the results attained with the standard ration (277). A supplement of 5% groundnut meal, 9% dried yeast, and 4% fish solubles, giving a total crude protein content of 17.2%, was equal in performance to the standard white fish meal supplement, however.

It has been confirmed (278) that all-plant rations containing barley, wheat feed, grass meal, and 13 to 16% groundnut meal (total protein 18.4 to 18.8%) give 90 to 93% as good growth, and only slightly inferior feed conversion efficiency, as similar rations containing 3.6 to 7.0% white fish meal (total protein 14.5 to 15.5%). Lysine rather than vitamin B₁₂ deficiency is responsible for the inferiority of groundnut meal. On high-energy barley-groundnut plant rations, however, growth and feed conversion efficiency were improved by additions of vitamin B₁₂ (1.4 γ /lb.). In general, all-plant rations are less palatable than those containing fish meal, and more energy is thereby wasted at the trough (275, 278). On barley-based rations (15% total protein) fed ad libitum, pigs receiving a groundnut-fish meal (3:1) supplement consumed more and grew faster (1.44 lb./day) than those supplemented with groundnut meal only (1.26 lb./day), but feed conversion efficiencies were equal (2.9) (278). This elevated consumption was not observed, however, when wheat feed replaced part of the groundnut meal and barley, but this may have been due to a seasonal effect on appetite. Carcasses of pigs fed all-plant rations after weaning to bacon weight were equal in size and quality to those which had received fish meal to 100 lb. weight.

In controlled feeding trials (279) similar to the foregoing, on rations of 17% crude protein containing barley, wheat bran, lucerne meal, and groundnut meal (15%), procaine penicillin, at the rather high level of 18 mg./lb., effected small but significant improvements in the growth and feed conversion efficiency of weanling pigs, but had little effect beyond 90 lb. live weight. The addition of vitamin B₁₂ (10 γ /lb.) conferred no further advantage. It was

* R. E. Evans, private communication.

also observed that the withdrawal of the penicillin after 90 lb. live weight had no deleterious effect on subsequent progress. Evans concludes that, on all vegetable diets, the antibiotic exerts its effect primarily as an appetite stimulant. Further studies are needed to discover whether greater responses to penicillin with rations containing groundnut would result under conditions approaching ad libitum feeding.

c. Ruminants

Groundnut meal is used as a range supplement for beef cattle and in commercial and farm-produced mixed feeds. Digestibilities (see also Table XII) and data on nitrogen utilization (280–283) have been recorded but are of doubtful significance for the wide range of rations incorporating groundnut meal. In Britain, Woodman (130) recommends 1 to 2 lb. per head daily as a supplement to basal rations of cereal, root crops, and roughage for mature beef cattle to be fattened at about 2 lb./day, but not more than 1 lb. per head, for example, for younger wintering stores. This contrasts with far more extravagant allowances in former years and with practices in the Peanut Belt in the United States where the amount fed ranges from 1 to 2 lb. (284) up to 6 to 10 lb./day (118).

Comparative feeding trials on fattening beef cattle in the United States have demonstrated the approximate equivalence of groundnut and cottonseed meals for growth (283, 285, 286). For fattening yearling (287) and 2-year-old steers (288), groundnut meal was slightly inferior and less palatable than cottonseed but was preferred in one case (287) for superior carcass quality. Again, groundnut meal (2 lb./day, supplementing milo and roughage) gave a slight advantage in gain, sleeker coats, and equally good carcasses, in spite of lower palatability (289). Daily gains of 1.9 lb./day have been reported for yearling Herefords fed corn-sorghum silage-groundnut hay and groundnut meal (290).

The rate of feeding groundnut cake or meal to beef cattle depends therefore on its price in relation to competitive feedstuffs, the required rate of gain, and the anticipated value of the carcass.

Groundnut meal is likewise a valuable constituent of dairy rations and is capable of sustaining high milk yields. As much as 20 lb./day has been used, though it is well to restrict feeding to younger stock (118). Four pounds per head are commonly fed in India (291). The manner of feeding of groundnut meal to different breeds to achieve various levels of milk production is computed from recommended allowances (130). Comparisons of groundnut, cottonseed, soybean, linseed, and gluten meals (292–297) have revealed only small differences as protein supplements for dairy cows. Groundnut meal had a “biological value for milk production” of 50% compared with 46 to 64% for other vegetable proteins (298). Insignificant differences in live weight

and milk and butterfat yields were observed in superficial comparisons of pressed and solvent-extracted meals (299–300). Rations containing appreciable quantities of groundnut meal have been used in diverse aspects of dairy research (301–303). Substitution of groundnut protein by urea did not favor production (302, 303).

Groundnut meals have long been used successfully in rations for horses and mules (1 to 1.5 lb./day) and for sheep (up to $\frac{1}{2}$ lb./day), but reports on performance are restricted (8, 131). Nutritive values for lambs (304) and a brief comparison of urea and groundnut meal for ewes (305) have been reported. "Biological values" for sheep have been determined (281, 306), and it has been shown that utilization of groundnut protein by Merino wethers is improved by the addition of starch to a low-energy wheat straw basal diet (306).

The need for a new approach to the use of groundnut and other oilseed meals in ruminant, particularly dairy, feeds has been made manifest by recent fundamental studies on protein utilization (307). Nitrogen balance experiments on sheep revealed poor utilization of casein supplements resulting from extensive and wasteful production of ammonia by rumen microorganisms (308). The ammonia is rapidly absorbed from the rumen and converted to urea and is not, therefore, available for microbial protein synthesis. Improved utilization was achieved by duodenal administration or by feeding heat-processed casein as hardened lumps. Rumen liquors from sheep fed groundnut meal had ammonia-nitrogen contents of 40 to 115 mg./ml., appreciably greater than those observed with casein, herring meal, and maize (309). This high rate of deamination was substantially reduced by the provision of readily fermentable carbohydrate such as starch. Practical studies on lactating goats showed the over-all nitrogen utilization (nitrogen in milk plus nitrogen in balance) from herring meal to be greater than that from groundnut meal; and for cows also, in a comparison of milk yields, herring was superior (310). The groundnut meals used in this work had high salt-peptizable nitrogen contents (75%), and it will be of interest to determine whether the relatively insoluble protein present in heat-denatured meals is subject to this excessive deamination.

Wasteful ruminal ammonia formation is of greater consequence in dairy rations in which protein requirements are relatively high. Further studies of nitrogen utilization and milk yield on rations containing differently processed meals, adequately balanced with readily fermentable carbohydrate, are needed, and until the fundamentals of protein nutrition in ruminants are better understood, groundnut meal in dairy feeds is best used in admixture with other oilseed meals (e.g., lin-

seed, soybeans, cottonseed) together with a small proportion of animal protein.

5. Use in Human Foods

This section is concerned only with general aspects of the properties and uses of groundnut meal as human food. The subject has been reviewed elsewhere (311, 312), and an account of the present and potential roles in human dietaries appears in Chapter 9. A discussion of possible use of isolated protein in human food is given in Chapter 11.

Cake made in crude small presses, such as the Indian ghani, and containing high residual oil, is consumed as food in China, India, Indonesia, Nigeria, etc. Local culinary practices in making confections are manifold. In Indonesia, for example, much of it is converted to "ontjom," nutritious white, orange, or red products made by treatment of the cake with fungi; in West Africa the cake is fried in groundnut oil.

High-quality flour is easily digested and reasonably well assimilated. For adults, its protein has a digestion coefficient of 99% and a biological value of 56 to 87% (162, 313). Poor tolerance involving digestive disturbances is attributed to the use of low-grade seed containing coarse particles of husk, dirt, and sand, and to the deleterious effects of skin and germ on the development of rancidity during storage. Rancidity during storage can be minimized by the use of antioxidants such as butylated hydroxyanisole or by admixture with maize products (314). Screw-pressed flour can be stored at 22° to 37° for 155 days in gunny bags without serious deterioration in flavor and nutritive value (315). High levels of intake (200 g./day) can give rise to clinical symptoms, and levels of 100 g./day have led to fatigue and loss of appetite (215). Nevertheless, at lower levels, groundnut flour comprises a useful supplement which increases the growth rate and general health of undernourished children (316, 317).

Therapeutic uses include the treatment of advanced pellagra (19) and glossitis (318), and it may well prove of value in sparing dried milk in the treatment of kwashiorkor.

Groundnut flour can be used in diverse foods—confections, soups, sauces, puddings, and bakery products (167, 319). It is used in admixture with wheat flour, preferably in small proportions (167, 311). Blends of groundnut and tapioca flour (1:3) have been investigated extensively in India (315, 320, 321), including round grains developed as a rice substitute (322). A dough is granulated and lightly roasted at 93° to 99° for 10 minutes in order to achieve an acceptable color, flavor, and shape. The optimum conditions for the process are rather critical; when operated properly it yields a product which can be cooked in 10 minutes. The grains are best stored (323) in cloth bags at 7 to 11% moisture, at relative humidities under 67%, conditions which favor keep-

ing well for 8 to 10 months. The nutritive value of the synthetic grains is increased by coating with calcium caseinate (321). Improvement and further development of synthetic grains in India is under way. Special tonic foods containing defatted groundnut flour have also been formulated, and their nutritive value has been checked with growing and breeding rats (324).

VIII. GROUNDNUT BY-PRODUCTS

The compositions of hay, shell, skin, and germ are summarized in Table XIII (325–327). The rather wide ranges in fat content are due

TABLE XIII
COMPOSITION OF GROUNDNUT BY-PRODUCTS^a

Component	Hay %	Shell ^b %	Skin %	Germ ^c %
Moisture	7.8–15	9.0–12	5.4–9.0	3.5–5.0
Crude protein (N × 6.25)	7.7–13 (64) ^d	5.0–9.4 (24–52)	11–18 (25)	26–38
Fat	1.1–5.1 (45–62)	1.2–4.0 (13–84)	1.2–28 (92)	42–46
Nitrogen-free extract	39–51 (78)	11–24 (11–60)	37–42 (16)	14
Crude fiber	22–34 (52)	58–79 (20)	8.0–21	1.6–2.5
Ash	9.7–17	2.8–8.8	2.1–3.3	2.7–3.1
Insoluble ash	4.4–7.7	0.4–1.9		
Total digestible nutrients	43–58	19–33	60–68	
Starch equivalent	49			
Mineral constituents:				
Sodium		0.16		
Calcium	1.4	0.32	0.38	0.072
Magnesium	1.0	0.24		0.21
Chlorine				0.015
Sulfur		0.06		0.18
Potassium	1.0	0.95		0.81
Phosphorus	0.15	0.07	0.06	0.60
Iron		0.0038	0.0065	0.0035
Copper		0.0047	0.013	
Nutritive ratio ^e	1:8.0	1:5.9	1:14	

^a General references: N. J. Morris and F. G. Dollear, *U.S. Dept. Agr., Bur. Agr. and Ind. Chem.* **AIC151** (1949); J. D. Guthrie, C. L. Hoffpauir, M. F. Stansbury, and W. A. Reeves, *ibid.* **AIC61**, pp. 50–78 (Rev. 1949); C. L. Hoffpauir, *J. Agr. Food Chem.* **1**, 668 (1953); H. E. Woodman, *Ministry of Agr. & Fisheries Bull.* **48**. H.M. Stationery Office, London (1954); B. H. Schneider, "Feeds of the World." West Va. Agr. Expt. Sta., Morgantown, 1947; E. Grewe, *Food Research* **10**, 28 (1945); G. S. Fraps, *Texas Agr. Expt. Sta. Bull.* **222** (1917); C. F. Huffman and C. W. Duncan, *J. Dairy Sci.* **35**, 30 (1952); T. F. Clark and E. C. Lathrop, *U.S. Dept. Agr., Bur. Agr. and Ind. Chem.* **AIC352** (1953).

^b Shell carbohydrates include reducing sugars 0.59%, disaccharides 1.7%, starch 0.74% pentosans 18%, lignin 25%, α -cellulose 25%, and uronic acids 6.9%.

^c Germ contains 7.9% reducing sugar.

^d Data in parentheses are digestion coefficients for ruminants.

^e See F. B. Morrison, "Feeds and Feeding," 22nd ed., p. 41. Morrison, Ithaca, New York, 1956.

to varying degrees of separation of kernels or fragments from the other components.

1. Hay

With care in handling and curing, high-grade, bright-colored hay can be produced from groundnut vines, which is both palatable and consumed economically by livestock. Dark, low-quality hay (due to rough handling, loss of leaves, and weather damage) is used as fertilizer and dirty hay should not be fed to horses or mules (131). Yields of hay from different varieties have been reported (55, 72, 328).

Ground peanut hay is marketed in the United States, and a dairy feed containing hay, wheat bran, cottonseed meal, snapped corn, and salt has been produced (19). Most hay is consumed locally, however, in combination with or in place of other legume hays, alfalfa, and silage. In an ear corn-velvet bean-cottonseed meal ration, sorghum silage, cottonseed hulls, and peanut hay were approximately equivalent as roughages for fattening steers (286). Fine grinding of peanut hay increased growth from 1.77 to 2.00 lb./day (290).

Good peanut hay is reported equivalent to soybean hay for dairy cattle (329). Sulfur dusting of vines does not affect its feeding value (330), and it is readily consumed even if darkened somewhat by frost damage (331). If made from whole plants including pods, feeding of hay to high-yielding Friesians should be limited to avoid purgative effects (105).

A preliminary comparison of green grazing and feeding hay to fattening swine has been carried out (332). On rations containing corn, peanut meal, and tankage, supplemented with minerals and vitamins, pigs fed peanut hay made gains almost equal to those on pasture for the range of 60 to 190 lb. live weight.

2. Shell

Groundnut shells (hulls) amount to between one-third and one-quarter of the weight of the whole pod. Though valued in some countries as a fuel and as a low-grade fertilizer, shells are among those agricultural residues for which more profitable uses are constantly sought in the United States (327) where large quantities tend to accumulate at shelling plants. Their relatively low value as fuel [8850 B.T.U./lb. (327)] imposes a restriction to local use, but in order to ease disposal problems many shelling plants do burn them (19). Large amounts of shell find local use as fertilizers to which inorganic nitrogen should be added. Commercially, they are ground and serve as fillers in fertilizers to improve flow characteristics.

Shells find application as cattle bedding, having a water-absorbing capacity equivalent to sawdust and shavings (131), though their value is offset somewhat by easy attachment to udder hairs and their propensity to attracting flies. Peanut hull litters are particularly suitable for broiler production (333, 334).

Groundnut shells are of low nutritive value containing over half their weight of crude fiber. They are used to adjust protein levels of meals and can be included in ruminant feeds as roughage (326, 335). Special products include molasses feed (80% hull) and molasses peanut feed (15% protein containing peanut screenings) (19).

Other outlets for shells (some of which are in commercial operation) include hard board (19, 336), cork substitute (327), floor-sweeping compounds, linoleum, explosives (19), destructive distillation (8), as a source of solvents (8), cellulose (8), and activated charcoal (337, 338), and as a filler base in insecticides and pesticides (327, 339).

3. Skin

The testa (2 to 4% of the seed) is removed during blanching in nut salting and butter production, in the manufacture of groundnut milk, in the production of meals for human consumption, and for protein isolation. Its thiamine (72, 340) and copper (341) contents are notably high—0.004 to 0.008% and 0.009 to 0.014%, respectively. The red-brown pigments consist mainly of 7 to 15% tannins (342, 343), and up to 3% phlobaphenes (342, 343), together with small amounts of leucoanthocyanins (343–345), flavones (343), and a flavanone (346). Cream skins contain very little tannoid. The quantity of skin pigments has been used as an index of the skin content of meals (347) and the groundnut flour content of a baker's premix (348).

The skins are relatively valueless, being rather bitter, but they can be included at low levels in ruminant feeds. The oil present is usually high in free fatty acid (about 50%). Apart from the oil, the digestibility of skin constituents is low and the feeding value correspondingly poor. Unless it is fine and indistinguishable, pigs do not feed well on rations containing skin (349).

4. Germ

The germ, or heart, forms 2 to 3% of the mature seed; it is generally removed along with small kernel fragments in peanut butter manufacture because it contains as yet uncharacterized bitter principles and rancidity promoters. In gross composition it resembles the meats but is slightly lower in oil. The fat-free germ contains non-protein nitrogen (0.42%) (21), choline (0.2%) (75), thiamine (5.6 γ /g.) (72), and phytin (0.5%) (22), and is lower in proteolytic activity than the cotyledons (49).

The germ can be crushed for oil and the meal residue or, more often, the whole germ finds application in small proportions in animal feeds. In its roasted form it is more palatable.

In a preliminary swine-feeding trial the germ was approximately equivalent to the whole kernel (349). Recently, in a microbiological assay of protein quality (194), a relative nutritive value of 98 has been reported for groundnut germ protein, compared with 52 for the protein of whole groundnut meal (casein = 100). It will be of interest to see whether this marked superiority of germ protein emerges from the results of other assay methods.

IX. TRENDS IN PRODUCTION AND UTILIZATION OF GROUNDNUTS AND GROUNDNUT PRODUCTS

World production of groundnuts may be expected to continue its upward trend, since expansion of groundnut cultivation figures prominently in the agricultural plans of India and China, the major producers. During the last two decades, however, there have been sharp changes in the pattern of international trade. Exports of seed are 60% less than prewar and, with increased crushing in growing areas, the proportion traded as crude oil has risen appreciably. There has been a decline also in exports of cake and meal, supplies of which vary greatly in quantity and quality. Future trends will be governed largely by the agricultural and economic policies of French West Africa, Nigeria, and India.

Yields of groundnuts per acre have increased in the United States, remained steady in West Africa, but decreased in India (2, 10). Progress in improving yields has been retarded in some areas by the persistence of primitive agricultural methods and failure to appreciate the value of thorough land clearance, crop rotation, and the use of artificial fertilizers, when available (350). Despite considerable research in selection and hybridization trials (17), improved varieties (72, 351, 352) yielding some 2000 lb./acre when grown experimentally, have been slow in entering commercial fields. Irrigation schemes and the introduction of more prolific and disease-resistant varieties, however, will improve yields slowly, and better overland transport facilities will reduce post-harvest losses due to crude methods of decortication and damage by weather, microorganisms, and insect pests. In the interim, fumigation with methyl bromide would seem to be of value in reducing losses due to insect damage during prolonged storage (353, 354).

Groundnut production entails heavy labor requirements. Progress in the mechanization of cultivation and harvesting is most advanced in the United States (355-357), where tractors and combine harvesters have been introduced, but results in Africa have been disappointing, particularly in the East African Groundnut Scheme of the United Kingdom (358). Rapid artificial curing by mechanical drying has proved difficult owing to skin slippage and kernel breakage (359-361), and, as yet, slow curing in well-constructed stacks produces peanuts of superior flavor.

Though highly nutritious, peanuts are by no means an essential article of diet in the United States. Peacetime consumption of processed peanut foods amounts to some 300,000 tons annually, and agricultural programs seem to be aimed at stabilizing production at about twice this level. This contrasts with West Africa and India, where increased pro-

duction and consumption of groundnuts as food offers the best prospect of raising the protein intake of undernourished millions. Whether this can best be achieved through the widespread use of products such as groundnut milk and flour will depend on the efficient and economic development of new processes at present in the pilot-plant stage.

In Europe the commercial development of groundnut protein isolate to replace casein as a paper coating adhesive is unlikely on account of its high cost and inferior color, but synthetic fiber production may well compete for supplies of groundnut meal at present used for feed. A plant in Scotland manufacturing Ardil, a groundnut protein fiber, has a production capacity of 10,000 tons per annum. The dried spent meal residue (containing 15 to 20% protein and 12% fiber) is used in cattle feeds, but no use has yet been discovered for the non-protein soluble fraction of the meal. If further improvements in the color and strength of the fiber were to raise its annual potential to 100,000 tons, economic and technical problems (24) in the supply of large quantities of light-colored, undenatured meal would possibly best be resolved by manufacturing the isolate in a groundnut-producing area.

Although groundnut meal has been utilized in a wide range of practical rations for all classes of poultry and livestock for many years, there remain significant gaps in our knowledge of its composition and nutritive properties which have been studied less extensively than those of soybean and cottonseed meals. There would seem to be a particular need for more comprehensive fundamental and applied research than hitherto on the following topics: the effects on the quality of groundnut meals of the variety and environment and pre- and post-harvest damage of the parent seed; the nature and extent of the effects on groundnut meal of oil-milling, with a view to the controlled manufacture of separate products of optimal nutritive value for monogastric and ruminant feeds; and the supplementary relationships of groundnut proteins and those of cereals, other oilseed meals, and animal protein concentrates. With such information, processors and users will be the better equipped to promote increased efficiency in the utilization of groundnut products in foods and feeds.

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CHAPTER 17

COTTONSEED MEAL

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I. INTRODUCTION

The fruit of the cotton plant, genus *Gossypium*, is a boll of seed cotton, i.e., cottonseed with the staple cotton lint fibers and cotton linters. Cotton lint is the principal economic product, yielding 75 to 85% of the total value of the products of the plant. In terms of weight, however, more seed than lint is produced. For every bale of 500 pounds of lint produced from seed cotton there are produced approximately 825 to 850 pounds of cottonseed which is a source of cotton linters, edible oil, hulls, and meal. Every ton of seed (2000 pounds) yields approximately 320 to 350 pounds of oil and 930 to 960 pounds of meal. Over 18 million tons of cottonseed were produced in 1956.

The history of cottonseed is, of course, derived from the history of cotton. Doubtless wild cottons, such as those known in India by 3000 B.C. and mentioned in a Hindu hymn of the Rig-Veda of about 1000 B.C., were first used (1*a*). But cotton is indigenous to many parts of the world: Africa, Arabia, India, Australia, and Central America (1*b*, 1*c*). Cotton was discovered by Columbus in the West Indies. Everywhere between the parallels of 40°N. and 40°S. latitude, with the exception of the extensive region of the United States now known as the cotton belt, cotton, either in its wild or cultivated state, was known and used at the date of the settlement of America (2).

No doubt cottonseed also found some uses among ancient peoples, but there is little available record. Pandit (3), writing in 1914, stated that cottonseed oil was quite familiar to the Indian from ancient times: "The old medical books of the Hindus mentioned the importance of cottonseed oil for external applications and the method is described of extracting it out of cottonseed. The method used consisted first of pounding the seeds and then boiling the pounded contents to produce a crude oil." Ward (4) mentions that Alexander of Macedon took with him the

products of cottonseed for his armies as they pushed eastward into Asia Minor, and Ghengis Kahn pressed cottonseed to supply oil for his men and meats for his animals on his expeditions in the Caspian Sea region and in China. Granted that there were scattered instances of crushing cottonseed for oil and meal from the beginnings of the cultivation of the cotton plant, these were probably infrequent and the products unimportant compared to the fiber itself. Most likely cottonseed was primarily a waste product which, if used at all, was spread as a fertilizer or fed directly to cattle.

The beginnings of cottonseed as an oilseed date with the invention of the gin by Eli Whitney in 1792. Although crude gins were known as far back as 300 B.C. (5), these were primarily for Egyptian-type cotton, for which the separation of the lint from the seed is much easier than for the so-called Upland cotton, the principal type grown in the United States. Development of the gin brought about for the first time the collection of large quantities of cottonseed in one spot, thereby intensifying the problem of disposal of a waste product and enhancing the opportunities for commercial operations on the seed.

Attention was drawn to cottonseed as a source of oil in 1783 when the London Society of Arts offered a gold medal on behalf of a British West India planter for extraction from cottonseed of a ton of oil and preparation of a hard, dry cake for cattle feed. This offer was not met, apparently because it required the production of such a sizable quantity (6). Commercial processing of cottonseed progressed more rapidly in Europe than in the United States, probably because of the predominance of Egyptian-type cottonseed. England and France were leading processors of cottonseed in the world in the nineteenth century; until 1880, England was crushing 200,000 tons of cottonseed annually. But as late as 1867 only four cottonseed mills were in operation in the United States; 300 were erected from that time until 1897. Only 40% of the crop was processed in 1897, and most of the rest was wasted. By 1927, 80% of the cottonseed produced in the United States was crushed. Additional historical information is given by Dougherty (7, 8), Nixon (9), and Curtis (10).

Cottonseed represents an outstanding example of conversion of an agricultural waste product and nuisance into an article of commerce, a source of valuable oil, protein, and other by-products. There are many parts of the world yet in which cottonseed either is a waste product or is used to little advantage, as fertilizer or fed directly to cattle. These areas, however, are shrinking in size and importance as the value of the oil and the need for good-quality vegetable protein are recognized.

II. PRODUCTION AND TRADE

1. World Review

a. Market Types

The world supply of cotton lint and seed may be divided into several market types: American Upland (*Gossypium hirsutum*), grown chiefly in the United States, Mexico, Central America, Brazil, Argentina, Turkey, Uganda, Israel, and Syria; Asiatic (*G. arboreum* and *G. herbaceum*), grown chiefly in India, Pakistan, and China; Egyptian, believed to be selections from *G. arboreum*, *G. herbaceum*, and *G. barbadense*, grown chiefly in Egypt, the Sudan, the United States, and Peru; Peruvian (*G. peruvianum*), grown chiefly in Peru; and Sea Island (*G. barbadense*), grown chiefly in the West Indies. Of the total production (exclusive of U.S.S.R. and China), American Upland supplies about 80%, Asiatic about 11%, Egyptian 6.4%, Peruvian 1.2%, and Sea Island 0.2%. Perennial tree cottons grown chiefly in Northern Brazil, Colombia, Ecuador, and Haiti still supply nearly 1% of the total production (11-16b).

b. Areas of Production

In the interval 1935-55, the total area planted in cotton has varied from a low of about 63.5 million acres in 1948 to a high of about 82.8 million in 1951 (17). Approximately three-fourths of the total cotton-producing area is located in five countries—India, the United States, China, Brazil, and the Soviet Union. Local conditions in some countries have led to acreage restrictions. For example, the great need for additional land for food crops in India led to restrictions on cotton and a decline from about 20 million acres in 1941 to 11 million in 1948. After the lifting of these restrictions the area under cotton gradually increased to over 17 million acres in 1953 (18a). In the United States, oversupplies of fiber have also led to acreage restrictions; cotton is one of the crops included under a price support program, and, if growers accept the program, acreage restrictions are officially imposed when considered necessary. Under this program the area under cotton was reduced from about 27 million acres in 1951 to about 16.9 million acres in 1955. These decreases by the major producers have been partially offset by increases in many of the smaller countries.

c. Production and Utilization

As shown in Table I, the world production of cottonseed during the period 1935-56 varied from a low of about 12 million tons during the

years 1945-49 to 18 million tons in 1956. During the years 1935-39, the first seven countries listed in the table—the United States, Russia, China, India (including Pakistan), Egypt, Brazil, and Mexico—supplied about 90% of the world total. The other six—Argentina, Peru, Turkey, the Anglo-Egyptian Sudan, Uganda, and the Belgian Congo—

TABLE I
COTTONSEED PRODUCTION—YEAR BEGINNING AUGUST 1 (1000 short tons)^a

Country	1935-39 ^b	1945-49 ^b	1950 ^b	1951 ^b	1952 ^b	1954 ^b	1956 ^c
United States	5,554	4,873	4,105	6,286	6,190	5,702	5,423
Soviet Union	1,640	1,117	1,680	1,918 ^d	1,958 ^d	—	2,880
China	1,593	1,086	1,361	1,680	1,624	1,652	1,736
India	2,984	1,290	1,523	1,764	1,683	2,184	2,274
Egypt	1,007	778	937	890	1,093	853	797
Brazil	935	649	792	936	749	768	—
Pakistan	^e	522	625	683	778	635	714
Mexico	160	277	552	611	600	854	859
Argentina	147	218	240	296	293	255	—
Peru	202	164	215	229	240	270	—
Turkey	126	137	276	306	352	306	—
Anglo-Egyptian Sudan	132	131	236	152	206	218	—
Uganda	143	116	147	161	136	153	—
Belgian Congo	87	99	100	122	106	120	—
TOTAL ABOVE	14,710	11,457	12,789	14,116	14,050	13,970	—
WORLD TOTAL ^f	15,295	11,940	13,480	16,945	16,920	18,565	18,320

^a Prepared with the advice of R. Hall, Washington, D. C.

^b U. S. Dept. Agr., *Agr. Statistics* 1955, Table 158; *ibid.*, 1954, Table 157.

^c Preliminary, U. S. Dept. Agr., *Foreign Agr. Service Circ.* FFO 13-57 (1957).

^d Not included in totals for year ending March 31. *Indian Central Oilseeds Committee, 8th Ann. Rept. For the year ending March 31, 1955. Oilseeds Ser. 46*, Hyderabad (DN), September, 1955.

^e Included in total for India.

^f World total exclusive of U.S.S.R. is given as 15, 15.4, and 15.2 million short tons for the years 1954, 1955, and 1956 respectively. *Food and Agr. Organization U.N. Monthly Bull. Agr. Economics & Statistics* 6 (4), 12 (1957).

supplied an additional 6 to 7%. By 1952 the combined production of the first seven countries had decreased to about 83% and that of the second group had increased to about 13% of the total. The cotton crop has increased enormously during the past twenty years in many of the smaller countries not listed in the table. For example, the three Central American countries (El Salvador, Guatemala, and Nicaragua) produced about 3000 tons of seed (calculated from lint production) in the years 1934-39 and about 119,000 tons in the 1954-55 season (15).

From available information it is estimated that about 75% of the world's supply of seed is processed to obtain oil, meal, and lesser amounts of linters and hulls. Only a few countries publish official statistics on the utilization of cottonseed. In Egypt and the United States (18b) from 85 to 90% of the seed is processed. About 60% of the Uganda seed is processed, and varying unspecified amounts are processed in other countries (17). India and probably China process the lowest percentages. Oil mill operators in India find it difficult to extract oil from cottonseed with the equipment available; moreover, Indian farmers consider whole cottonseed to be superior to cottonseed meal for cattle feeding. For these reasons only about 5% of the seed is processed, and the remainder is used for planting, for cattle feed, and for fuel (18a).

Recent information is not available on cottonseed utilization in China and the Soviet Union. Judging from earlier reports there are relatively few modern processing plants in China and a relatively large number of modern plants in the Soviet Union.

d. Exports and Imports of Seed and Meal

Unlike other oil-bearing seeds such as soybeans and flaxseed, the major portion of the world supply of cottonseed is consumed within the country of origin. Trade in cottonseed (in the period 1935-55) reached a high point in 1938. Egypt, China, Brazil, Argentina, India, and the United States were the chief exporting countries; and the three major importing countries were Denmark, Japan, and the United Kingdom, whose combined imports were over 700,000 tons. During World War II, exports decreased rapidly and have never regained the 1938 level. Total imports in 1953 were 322,000 tons; of this amount the Anglo-Egyptian Sudan supplied 118,000 tons, with Nigeria, Uganda, Mozambique, Nicaragua, Syria, and Turkey supplying from 20,000 to 30,000 tons each. Chief importing countries were the United Kingdom, Germany, Greece, Japan, and Lebanon. Throughout this period the United Kingdom was the leading importer of cottonseed for crushing. Imports ranged from 620,000 tons in 1938 to 168,000 tons in 1953 (17). Prices in the United Kingdom in 1955 were about £100 per long ton of oil, £27 per ton for screw-pressed cake with 5% oil, and £32 per ton for cake containing 40% crude protein (18c). Most of the processing is by screw press on undecorticated seed. Only 20% or so of the seed is decorticated.

The increasing need for edible oils in many cotton-growing areas makes it seem probable that local demands will reduce still further the amount of seed available for export.

2. Major Producers

a. United States

Cotton production in the United States is centered in fourteen states extending from the Atlantic Coast to the Pacific Coast. During the period 1920-55, cotton growing has been shifting from the rolling Piedmont country and the rolling lands of the south-central states to flat lands in the Mississippi, the Rio Grande, San Joaquin, and Imperial valleys, and the flat valleys of Arizona. These lands are more suitable for tractor farming than are the rolling lands, and, for the most part, yields per acre are higher in the flat lands, particularly those with plentiful rainfall, and in the irrigated valleys of the West and Southwest (19a).

Statistics on lint and cottonseed production in the United States are shown in Table II. Cotton acreage varied from a high of about 27.8 million acres in 1935-39 to a low of 16.9 million acres in 1955. During this same period the average yield of lint per acre increased from 226 to 417 pounds and the yield of seed from 398 to 713 pounds. About 83% of the seed was processed in 1935, and 92% in 1954. The farm value of the lint was approximately five times that of the seed in 1935; in later years this ratio was increased.

Recoveries and values of crude oil, cake and meal, hulls, and linters per ton of cottonseed are shown in Table III. Installation of larger and more efficient processing plants has resulted in a gradual increase in meal recovery from about 44 to 48%; recoveries of all products increased from about 92% to over 95%. Prices have varied over a wide range. In earlier years, the value of the oil was about twice that of the meal, but in recent years oil prices have decreased while meal prices have remained approximately constant. The total value of products recovered per ton of cottonseed varied from a low of \$41 in 1935-39 to a high of \$136 in 1947.

Small amounts of seed are exported, chiefly for planting. In the years 1945-55, meal exports have varied from 10,000 to 125,000 tons; meal imports usually exceed exports.

b. China and the Soviet Union

The area under cotton in China ranged from 7.5 million acres in 1938 to 10.2 million in 1953. Seed production over the period 1935-55 has averaged about 1.6 million tons. The chief cotton-growing areas are the Yangtze and Yellow River basins and the Provinces of Chekiang and Yunnan in western China. Types of plants grown are *G. Nanking*, an annual or perennial bush type, and acclimated American Upland types.

The area under cotton in the Soviet Union ranged from about 5.1 million acres in 1938 to a low of 3.8 million in 1948 and another high of 5.1 million in 1953. Cottonseed production was approximately equal to that in China during the years 1935-49. Since then, production in the Soviet Union has increased to over 2 million tons. There are three important cotton-growing areas in the Soviet Union: Central Asia; Transcaucasia and the Stavropol; and the Crimea and the Azov-Black Sea section. The varieties of cotton grown are believed to be selections from *G. Nanking*, *G. herbaceum*, and acclimated American Upland cottons.

TABLE II
ACREAGE, PRODUCTION, AND FARM VALUE OF COTTON AND COTTONSEED IN THE UNITED STATES; AVERAGE 1935-39, ANNUAL 1945-55
—YEAR BEGINNING AUGUST 1^{a-c}

Year	Production				Farm value, unit price				Total value		
	Harvest 1000 acres b	Lint 1000 bales b	Seed 1000 tons c,d	Lint lb./acre b	Seed lb./acre c,d	Seed processed 1000 tons e,f	% production	Lint cents/lb. b	Seed dollars/ton c	Lint 1000 dollars b	Seed 1000 dollars c,d
1935-39	27,788	13,091	5,554	226.0	398	4,653	83.8	9.99	25.45	640,684	137,507
1945	17,029	9,015	3,664	254.1	430	3,262	89.0	22.52	51.10	1,014,823	187,155
1946	17,584	8,640	3,514	235.7	400	3,090	87.9	32.64	71.90	1,409,668	252,697
1947	21,330	11,860	4,682	266.6	439	4,082	87.2	31.93	85.90	1,892,949	402,058
1948	22,911	14,877	5,945	311.3	519	5,332	89.7	30.38	67.20	2,260,089	399,755
1949	27,439	16,128	6,559	281.8	478	5,712	87.1	28.58	43.40	2,304,636	284,810
1950	17,843	10,014	4,105	269.0	460	3,723	90.7	40.07	86.40	2,005,684	354,593
1951	26,949	15,149	6,286	269.4	467	5,476	87.1	37.88	69.30	2,868,720	435,891
1952	25,921	15,139	6,190	279.9	478	5,563	89.9	34.59	69.60	2,617,644	430,959
1953	24,341	16,465	6,748	324.2	554	6,256	92.7	32.25	52.70	2,654,683	355,300
1954	19,251	13,696	5,709	341.0	593	5,249	91.9	33.61	60.30	2,301,212	344,200
1955	16,928	14,721	6,038	417.0	713	5,588	92.5	32.40 ^g	44.60	2,382,342	269,000

^a Prepared with the advice of R. Hall, Washington, D. C.
^b U. S. Dept. Agr., *Cotton Situation* CS 164, Table 11, May 29, 1956.
^c U. S. Dept. Agr., *Agr. Statistics* 1952, Table 162; 1955, Table 155; years 1935-39, 1945-1952.
^d U. S. Dept. Agr., *Fats and Oils Situation* FOS 180, Tables 7 and 8, September 27, 1956.
^e U. S. Dept. Agr., *Agr. Statistics* 1952, Table 166; 1955, Table 159; years 1935-39, 1945-53.
^f U. S. Dept. Agr., *Fats and Oils Situation* FOS 180, Table 9, 1956; years 1954 and 1955.
^g Average price to May 1, 1956, including allowances for unredeemed loans.

TABLE III
COTTONSEED PRODUCTS IN THE UNITED STATES; AVERAGE RECOVERIES AND APPROXIMATE VALUES PER SHORT TON OF COTTONSEED;
AVERAGE 1935-39, ANNUAL 1945-55—YEAR BEGINNING AUGUST 1^{a-c}

Year	Crude oil				Cake and meal				Hulls				Linters				Totals			
	Recovery		Value		Recovery		Value		Recovery		Value		Recovery		Value		Recovery		Value	
	lb.	%	lb.	dollars	lb.	%	lb.	dollars	lb.	%	lb.	dollars	lb.	%	lb.	dollars	lb.	%	lb.	dollars
1935-39	b,e	d	b,e	b,d	b,e	d	b	b,e	b	d	b,e	b,e	b	d	b,e	b,e	d	d	b,e	b,e
1945	310	15.5	22.25	11.66	904	45.2	514	2.47	146	7.3	4.33	1874	93.7	40.71	1874	93.7	40.71	1874	93.7	40.71
1946	312	15.6	39.78	24.17	879	44.0	480	3.17	182	9.1	8.34	1853	92.6	75.46	1853	92.6	75.46	1853	92.6	75.46
1947	315	15.8	78.06	32.90	882	44.1	471	3.77	191	9.6	18.07	1859	92.9	132.80	1859	92.9	132.80	1859	92.9	132.80
1948	313	15.6	82.16	38.13	930	46.5	452	3.53	186	9.3	12.46	1881	94.0	136.28	1881	94.0	136.28	1881	94.0	136.28
1949	320	16.0	49.34	26.37	897	44.9	463	1.53	183	9.2	7.21	1863	93.1	84.45	1863	93.1	84.45	1863	93.1	84.45
1950	323	16.2	40.44	26.40	895	44.7	469	1.64	176	8.8	9.87	1863	93.1	78.35	1863	93.1	78.35	1863	93.1	78.35
1951	321	16.1	65.45	32.08	896	44.8	461	4.15	185	9.2	29.99	1863	93.1	131.67	1863	93.1	131.67	1863	93.1	131.67
1952	320	16.0	41.54	36.18	930	46.5	451	3.92	185	9.2	16.06	1886	94.3	97.70	1886	94.3	97.70	1886	94.3	97.70
1953	328	16.4	46.74	34.79	961	48.0	431	3.71	184 ^e	9.2	11.02	1904	95.2	96.26	1904	95.2	96.26	1904	95.2	96.26
1954	332	16.6	45.05	28.57	946	47.3	444 ^d	2.58	184 ^e	9.2	8.28	1906	95.3	84.48	1906	95.3	84.48	1906	95.3	84.48
1955 ^f	331	16.5	44.32	30.26	976	48.8	429 ^d	3.08	187 ^e	9.4	7.24	1923	96.1	84.90	1923	96.1	84.90	1923	96.1	84.90
	339	16.9	44.14	23.64	942	47.0	446 ^d	1.25	180 ^e	9.0	6.74	1907	95.3	75.77	1907	95.3	75.77	1907	95.3	75.77

^a Prepared with the advice of R. Hall, Washington, D. C.
^b U. S. Dept. Agr. Statistical Bull. 147, Table 26, 1954.
^c U. S. Dept. Agr., Fats and Oils Situation FOS 180, September 26, 1956.
^d Calculated from Official Statistics.
^e U. S. Dept. Agr. Weekly Linters Rev. September 14, 1956.
^f Preliminary figures.

c. India

About 90% of the total crop in India is of Asiatic species (chiefly *G. arboreum* and *G. herbaceum*) and the other 10% from American Upland cottons (*G. hirsutum*). In its original form the *arboreum* species is a perennial which grows to a height of 6 to 10 feet. Under cultivation, especially in areas with a short growing season, the perennial has evolved into an annual. There are about forty varieties of cottonseed grown in different parts of the country; new strains and varieties are being developed and popularized by the various agricultural research stations (18a, 19b). The principal sections growing cotton are Bombay, Hyderabad, Madras, and Punjab, with the Bombay section producing the largest tonnage.

About 5% of the total quantity of seed produced is processed; the remainder is used for planting and for cattle feed. There are about fifty oil mills in India; only six use modern processing techniques (chiefly screw presses), the others recover oil from the whole seed without removing the linters or hulls (18a).

Illustrative prices of seed and seed products in the Bombay area are as follows (18a):

Product	November, 1952	November, 1955
	Rupees per ton (2240 lb.)	Rupees per ton (2240 lb.)
Seed	225	210
Oil	1540	1014
Meal (cake)	168	225
Hulls	42	28
Linters	360	336

d. Egypt

Cotton is the principal money crop in Egypt and is grown throughout the entire Nile Valley wherever water for irrigation is available. The soils of both the valley and the delta are of alluvial origin.

At least three species of cotton (*G. herbaceum*, *G. arboreum*, and *G. barbadense*) have been known in Egypt since early times. Between 1820 and 1920 a number of varieties appeared which are believed to be selections from these original species. One of the first of these was the Jumel variety, followed by the Ashmouni, Mitafifi, Sakel, Zagora, Karnak, and Giza varieties. The Egyptian Cotton Research Board was established in 1920, and many selections and hybrids have been developed since then.

Because of the accumulation of cotton during World War II, production control programs were instituted in 1942 and are still in force (1956). As a result, the area planted to cotton was reduced from 1.7 million acres in 1941 to 725,000 acres in 1942. By 1949 the area planted had been increased to about 1.4 million. Yields of lint per acre averaged about 600 pounds in 1942 but decreased to about 500 pounds in 1945, chiefly because of lack of sufficient fertilizer (12).

Egyptian cottonseed is slick and produces relatively few linters. The seed contains about 20 to 21% oil, compared with about 14 to 19% in India and

about 19% in the United States. The hulls make up about 40% of the seed in Egypt as compared to about 49 to 56% in India and 36 to 50% in the United States.

About 87% of the seed is crushed. The seed is allocated to the oil mills by the Government at a price of 9.2 Egyptian pounds per ton (1955). Anglo-American hydraulic presses are used in the majority of the oil mills. In 1955 there were 606 hydraulic presses and 45 screw presses in the country; there was also one 200-ton prepress solvent-extraction plant. Uncorticated seed is processed in all the oil mills with the exception of the prepress solvent-extraction plant. Average recoveries and values per ton of decorticated and uncorticated seed are shown in the following table:

Product	Uncorticated seed		Decorticated seed	
	kg./ton	Egyptian pounds	kg./ton	Egyptian pounds
Salad oil	147	8.644	173.5	10.202
Slabcake or meal	810	5.589	446.5	5.135
Stearine	13	0.644	8.2	0.402
Soap stock	7	0.175	20.6	0.577
Hulls			331.7	0.497
Total value of products per ton		15.052		16.813

The export of slabcake was banned by the Government in 1942 to encourage its use in animal feed. Some of the cake is still used for fuel and fertilizer, but the major portion is now used for stock feeding (12, 20a).

e. Brazil

The area under cotton in Brazil has averaged between 6 and 7 million acres in the years 1935-55. Seed production has varied from 650,000 to 935,000 tons. São Paulo in south Brazil produces over 50% of the cotton; next in importance are Ceará and Rio Grande do Norte in northeast Brazil. Cotton in south Brazil is of American Upland origin, chiefly Stoneville 2B, Texas Big Boll, and Express. In north Brazil are grown some perennial tree cottons known as Serido and Sertao and an annual Upland type known as Mata (11, 13).

Most of the processing is in Marília, Bauru, and Presidente Prudente; approximately 500,000 to 525,000 tons of seed are processed annually. About 55% is processed by prepress solvent extraction, 20% by screw presses, and 25% by hydraulic presses. This proportion is expected to change to about 75% prepress solvent extraction on the basis of present trends.

The major portion of the meal contains about 36 to 38% protein, but in north Brazil the meal has a lower protein content. Meal is used both as a fertilizer and as a stock feed. Relative prices of seed, crude oil, and meal in 1950 were respectively, 0.70, 5.00, and 0.70 cruzeiro per kilogram; in 1955 these were 1.90, 15.00, and 0.70 cruzeiro per kilogram (20b).

f. Pakistan

The total area under cotton averages about 3 million acres; only about 60,000 acres (chiefly local varieties of *G. arboreum*) are grown in East Pakistan in two cotton-growing areas, the alluvial valley of the Ganges River in the West and the heavy clay loam soils of the East and North. Production in West Pakistan is concentrated chiefly in the provinces of Sind and West Punjab in the irrigated valley of the Indus River and its tributaries. Over 90% of the cotton is grown from acclimatized American Upland varieties, and nearly all the remainder from perennial varieties of *G. arboreum*.

Cottonseed production in 1954 was estimated to be about 635,000 tons. About 10% is used for planting, 15% is crushed, and 75% is used for cattle feeding. Farmers consider the whole seed to be superior to the meal for cattle feeding even though the Lyallpur experiment station has demonstrated the superiority of the meal for animal feeding.

In 1951 West Punjab had a total of 26 crushing mills; the industry is expected to grow as a result of the work of the experiment stations in explaining the greater value of the meal as a feed and also through the increasing demand for oil products (16a).

g. Mexico

Cotton grows wild in many parts of Mexico and is believed to be indigenous to the country. The major portion of the cotton land is irrigated and is located in the northern part of the country in the Districts of Laguna, Matamoros, Mexicali, Delicias, Sonora, Tamaulipas, and Sinaloa (11). The area under cotton increased from 642,000 acres in 1938 to 2.1 million in 1953. During the same period seed production increased from about 16,000 to 581,000 tons.

All cotton is of American Upland type, the chief varieties being Delta Pine, Acala 4-42, and Empire. Leading processing centers are in Matamoros and Tamaulipas. Both screw and hydraulic presses are used in processing, although the trend is toward more screw-pressing. The cake and meal are sold as protein concentrates for stock feeding (17).

The 1955 prices for seed ranged between 433 and 550 pesos per metric ton; meal (38 to 40% protein) ranged from 450 to 525 pesos per ton; and oil from 2.95 to 3.40 pesos per kilogram (21).

h. Other Producers

(1) *Turkey*. The area under cotton in Turkey increased from 680,000 acres in 1938 to nearly 1.5 million in 1953; seed production increased from 126,000 tons in 1935-39 to 325,000 tons in 1953. The principal cotton-growing area is Cukurova, around the Mediterranean Sea. In recent years Turkey has been exporting about 25,000 tons of seed annually. After the requirements for planting have been met, much of the remainder is processed and both the oil and meal are used within the country. Most of the processing is by screw press.

(2) *Argentina*. Cotton is grown for the most part in the subtropical Chaco, Santa Fe, Formosa, and Corrientes Provinces. About 8 million acres are said to be suitable for cotton growing. The area under cotton has increased from about 1 million acres in 1938 to 1.5 million acres in 1953. During the same period seed production increased from 147,000 to 324,000 tons.

The commercial cottons grown are almost all American varieties such as Delta Pine 12, Stoneville 2B, Acala, and Coker's. Leading processing centers are located at Resistencia, Barranqueras, and Sáenz Peña in the Chaco Province, and at Reconquista in the Province of Santa Fe. Processing is done with hydraulic presses for the most part, although some processing plants use screw presses and solvent extractors; the trend is toward prepress solvent extraction. In 1955 cottonseed sold at 400 to 420 pesos per metric ton; the oil (semi-refined) was valued at 4000 pesos per ton; and the cake sold for 250 to 260 pesos per ton, the solvent-extracted meal selling for less than 210 pesos per ton. Grain and pastures are so abundant that a relatively small amount of cottonseed meal is used for cattle feeding and the greater portion (about 95%) is exported to Europe. Compared with the processing of sunflower seed, linseed, and peanuts, the cottonseed processing industry in Argentina is relatively small (13, 22, 23).

(3) *Peru*. Cotton is grown chiefly in the following coastal river valleys—Piura, Casma, Huarmey, Huaura, Lima, Canete, Chincha, Pisco, Ica, and Nazca. The area under cotton in Peru was about 471,000 acres in 1938 and had decreased to 440,000 acres in 1953. Seed production during this period ranged from 202,000 tons in 1935–39 to 254,000 tons in 1953. Both Peruvian- and Egyptian-type cottons are grown. The Peruvian type known as Tanguis accounts for about 84% of the total; Egyptian types—Karnak and Pima—make up much of the remainder.

Processing centers are located in nearly all the river valleys. About 70% of the seed is processed with hydraulic presses, and the remainder with screw presses and solvent extraction. A typical cottonseed meal contains 39% protein and 6% oil. Under present regulations 60% of the meal and cake is set aside for local consumption and 40% is exported. The 1955 prices for seed, meal, and refined oil were 35, 16.8, and 280 sol per quintal (13, 21).

(4) *Sudan*. The area under cotton ranged from 458,000 acres in 1938 to 651,000 acres in 1953. The principal cotton-producing area is the Gezira, the large fertile plain between the White and Blue Niles. This area normally accounts for about three-fourths of the country's entire production. Other important cotton-growing areas are the deltas of the Gash and Baraka rivers and the Nuba Mountains of Kordofan. American-type cottons are grown for the most part in Kordofan, and Egyptian types such as Sakelardis in the remainder of the country.

Cotton production in the years 1935–39 averaged 439,000 bales, and in 1953 about 400,000 bales. Seed production in 1935–39 averaged about 132,000 tons, and 214,000 tons in 1953. Nearly all the seed is exported to Egypt and Europe for processing (12).

(5) *Uganda*. About 150,000 acres of cotton are grown in the basin of Lake Kyoga, on land which ranges in elevation from about 2000 to 3700 feet. The total area under cotton ranged from 1.2 million acres in 1938 to 1.6 million in 1953. Cotton production in the years 1935–39 averaged 281,000 bales, and in 1953, 320,000 bales. Seed production averaged 143,000 tons in the years 1935–39, and 169,000 tons in 1953. Outside the United States, this country is practically the only source of fine-fibered, long-staple Upland cotton; consequently, cotton production in Uganda is of importance in the world market even though the quantity produced is relatively small. The seed is essentially all crushed in Uganda or in neighboring countries, and the oil and meal produced are used within East Africa (12).

(6) *Central America*. Cotton production in Central America increased from about 8000 bales in the years 1934–38 to 290,000 bales in the 1954–55 season: El Salvador produced about 75,000 bales, Guatemala 40,000, and Nicaragua 175,000. Each country averaged a yield of about a bale per acre. American cross-breeds of cottonseed are used, chiefly Acala-1517 and DLP-15. Seed production calculated from bales of lint produced would amount to about 30,000 tons in El Salvador, 16,000 tons in Guatemala, and 75,000 tons in Nicaragua.

During the 1954–55 season the seed sold for 25 dollars per ton in Nicaragua and 30 dollars per ton in El Salvador and Guatemala. In El Salvador seed not used for planting is crushed; both screw and hydraulic presses are used. The press cake contains about 6% oil and is used for cattle feeding; and the oil is refined, supplying the major portion of the vegetable oil in El Salvador. The major portion of the seed produced in Guatemala and Nicaragua is exported (15).

III. APPEARANCE AND STRUCTURE

1. Principal Species and Conditions of Growth

Cotton, genus *Gossypium*, belongs to the sub-tribe *Hibisceae* of the natural order *Malvaceae* (16b). There are about 40 species of the genus *Gossypium*, the more important of which are *G. hirsutum* (American Upland), *G. herbaceum* (Asiatic), and *G. barbadense* (Egyptian). In its natural circumstances the cotton plant is a perennial shrub or small tree, but under cultivation it is treated as an annual. It thrives best in sandy soil and damp, humid regions that are near water, an environment especially characteristic of the southern United States and the river valleys of India and Egypt. In recent years, considerable cotton has been grown under irrigation, as in the western plains of Texas, and the valleys of California and Arizona. Only in a few sections of the world is cotton cultivated as a perennial, such as is done in Brazil where quantities of “tree” cotton are cultivated (24).

Cotton matures five or six months after planting and is ready for picking soon after ripening. The height of the plant varies with the species and variety, Upland varieties growing to a height of 2½ to 4 feet; some varieties grow as high as 8 feet.

2. Growth and Development of Seed

After fertilization of the flower, either by cross- or self-fertilization, the fruit matures in 40 to 60 days. The cotton fruit is a leathery capsule which when mature is called a “boll” (25).

3. Structure

It can be said that cottonseed is a product of the cotton plant and consists of two parts, the hull or spermaderm from which the staple

cotton lint and the cotton linters or fuzz arise, and the kernel or embryo from which the oil and meal are obtained. The seed is in the form of a pointed ovoid body between 8 and 12 millimeters in length; North American Upland cottonseed weighs about 32 pounds to the bushel (26). There are two kinds of hair on cottonseed, long hairs (staple lint) and short hairs (linters). Ginning is the process of removing the staple-length fiber from the seed cotton—cotton lint and seed as removed from the boll—leaving the cottonseed. Most species of seed contain short hairs as well as long hairs and are covered with these short cotton fibers

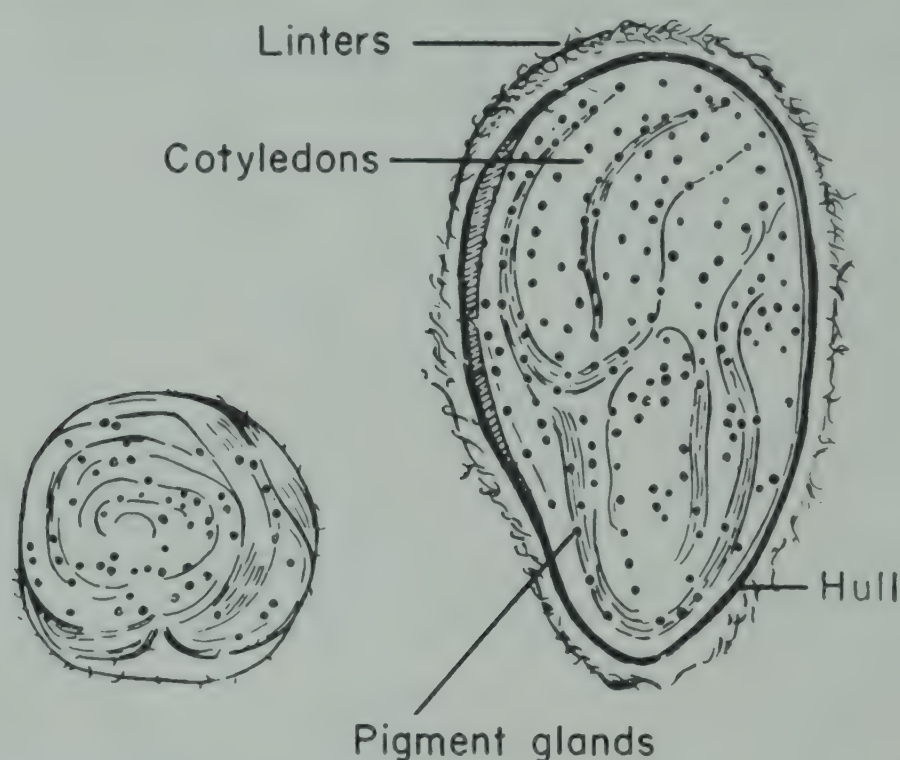


FIG. 1. Longitudinal and transverse sections of cottonseed, *Gossypium hirsutum*. (Drawing by Leah Katz.)

after ginning. Some species, notably Egyptian cottonseed, have no adhering linters and after ginning appear “bald or naked” and black in color.

Figure 1 presents sketches of longitudinal and transverse sections of the cottonseed. Details about the morphology and microscopic structure of cottonseed may be found in the literature (26–28a).

The two principal elements of the seed are the spermaderm and the embryo; and there is a third structure, a membrane, which completely envelops the embryo. Cottonseed is a dicotyledon, there being two large flat cotyledons and the so-called axial organs, the radical, the hypocotyl, and the epicotyl. The cotyledons are folded around the radical and over the top of the hypocotyl in the embryo.

Oil is found in all the tissues and organs of the embryo but is most abundant in the mesophyll of the cotyledons and the parenchymatous cells of the hypocotyl (27). It is embedded in the tissue in minute droplets. When sections are observed in water, the droplets can be seen to

coalesce, forming larger droplets. Protein occurs in aleurone grains and as a component of the cytoplasm. It is found in all the organs of the embryo.

4. Pigment Glands

Cross sections of the embryo disclose innumerable dark "specks." (See also Fig. 1.) These are pigment glands; they also have been referred to as gum or resin glands. They are distinct morphological structures which are relatively large, ovoid spherical bodies, 100 to 400 microns in length on the long axis. These glands, so-called, are characteristic of all species of the genus *Gossypium*. Their first appearance is in the 15-day-old embryo where they are distributed in the cotyledons and hypocotyls (28*b*). In the cotyledons they are directly beneath the palisade layer into which they often project.

These pigment glands have been investigated extensively by Charlotte Boatner and her associates; the results of their investigations are contained in a review and in a number of publications (29–32). [See also Stanford and Viehovever (33).] The gland wall is made up of five to eight closely fitting, thick, curved plates composed of materials containing cellulose, pectin, hemicellulose, and some unidentified uronic acid derivatives. Material within the pigment glands seems to be in a semiliquid state which under suitable conditions can be discharged into the surrounding medium. Pigment glands vary in color with growth and environmental conditions from yellow, orange, or red to purple.

Contact of pigment glands with water brings about an immediate discharge of their contents. Certain water-miscible organic solvents will also cause a discharge of the contents but at a slower rate. Solvents such as hydrocarbons, chlorinated hydrocarbons, and triglycerides are inert toward the pigment glands; they have no effect even after contact for 24 hours. It appears that the pigments contained in the glands will remain largely unaffected until the walls have been ruptured but will be extracted thereafter by any solvent in which they are soluble. Solvents which are themselves incapable of attacking the glands may effect rupture after prolonged contact because of their slight content of moisture (34).

Advantage was taken of the stability of pigment glands to develop a process for their separation from the remainder of the seed. By adjusting the density of a solvent mixture, the glands can be made to float where the other particles sink (35, 36). Application of this method has made possible preparation of pigment glands and gland-free meal, both valuable materials for research (37). Their availability has facilitated progress in gossypol chemistry and in understanding the role of pigments in the processing of cottonseed.

Most of the deeply colored pigments of the seed are concentrated in the pigment gland; and the principal pigment is *gossypol*, which may constitute from 20 to 40% of the weight of pigment glands. Other constituents of the pigment glands are not well defined. Pigment glands contain nitrogen, but since it is very difficult to separate them completely from meal fragments the amount of nitrogen that is contained within the pigment glands themselves is not known.

IV. COMPOSITION OF SEED

1. Proximate Composition

Cottonseed contains on the average 44.5% hulls and linters, and 55.5% meats (kernels). Delinted cottonseed contains 36% hulls and 64% meats (38). The meats contain about 7% moisture, 30% crude protein, 30% oil, 24% nitrogen-free extract, 4.8% crude fiber, and 4.4% ash (39). An idea of the range in composition, which depends on species, environment, and specific varieties, is given in Table IV. More information on the influence of variety and environment is given in a later section.

2. Major Constituents

a. Oil

Cottonseed oil is the major economic component of the seed (see Table III) and occurs in the seed in quantities approximately equal to the amount of protein. Along with other common fatty oils, cottonseed oil consists primarily of triglycerides of fatty acids. For this oil the major fatty acids are linoleic (approximately 50%), oleic (approximately 23%), and palmitic (approximately 23%). There are also small quantities of myristic, stearic, and palmitoleic acid (40). Cottonseed oil is liquid at ordinary temperatures, has good flavor and stability to oxidative rancidity, and, indeed, is one of the world's major prime edible vegetable oils.

It finds its major uses in the preparation of shortenings or as a constituent of margarine. If the oil is treated by a process called winterization whereby the triglyceride fractions which are solid at icebox temperatures are removed, it can then be used as a salad oil, and large quantities are sold for that purpose. In the United States, the approximate distribution of uses for the oil is: margarine, 23%; shortening, 29%; salad oils, salad dressing, and mayonnaise, 41%. Close to 1.5 billion pounds of cottonseed oil are consumed annually in the United States.

Aside from the glycerides, there are small quantities of other fatty materials, soluble in fat solvents. In actual practice substantial quantities of some of them may remain in the meal residue after oil extraction; hence they are also properly considered components of the meal. Among these minor constituents in the crude oil are phosphatides, 0.7

TABLE IV
COMPOSITION OF COTTONSEED

Source	Mois- ture	Oil	Crude protein ^a	Linters	Kernels in seed	Weight of 100 g. of seed	Refer- ences
	%	%	%	%	%	g.	
United States (American Upland)	9.0–12.8	18.2–19.5	18.7–21.3	10.4–11.4	50.2–63.3	11.1	b–d
India	6.7–10.7	13.9–19.4	16.8–20.9	9.2–12.8	44.4–50.8	4.8–8.6	e
Indian varieties	6.8–10.6	15.2–18.0	—	3.6–5.4	—	5.2–6.5	f
American varieties	9.0–12.1	16.4–18.3	—	12.3–13.2	—	9.0–10.8	f
Egypt ^g	7.8–9.5	20.0–21.0	17.1–19.8	—	—	9.1–9.6	h
Brazil (tree type)	6–7	20–22	19.1–21.2	1–2	55–56	—	i
Sudan	5	20.5	17.2	39.6 ^j	60.7	—	j

^a Nitrogen \times 6.25.

^b U. S. Dept. Agr., Agr. Marketing Service, "Cottonseed Quality, Crops of 1954 and 1955," 23 pp. (Mimeographed.) Memphis, Tennessee, October, 1956.

^c W. H. Tharp, in "Cottonseed and Cottonseed Products" (A. E. Bailey, ed.), Chapter 4. Interscience, New York, 1948.

^d J. D. Guthrie, C. L. Hoffpauir, M. F. Stansbury, and W. A. Reeves, U. S. Dept. Agr. Bur. Agr. Ind. Chem. AIC-61 (Rev. March, 1949).

^e M. N. Rao and S. Kuppaswamy, *Central Food Tech. Research Sta. (Mysore) Bull.* **2** (12), 303 (1953).

^f V. R. Harwalkar, K. T. Achaya, and S. A. Saletore, *J. Indian Chem. Soc. Ind. & News Ed.* **16** (2, 3), 87 (1953).

^g Consolidation of data on the following varieties: Karnak, Giza 30, Menoufi, and Ashmouni.

^h M. S. Montassir, The Nile Ginning Co., Minia, Egypt, private communication.

ⁱ R. T. Kinsey, Anderson Clayton & Cia., São Paulo, Brazil, private communication.

^j Unpublished data. Sum of linters and hulls.

to 1.8%; steroids, up to 1.6%; tocopherols, 0.1 to 0.14%; fatty acids, approximately 1% (will depend on harvest and storage conditions); gossypol and its degradation products, 0.3 to 3%; and small quantities of hydrocarbons, pigments, carbohydrates, proteins, and mucilaginous substances (41).

Soapstock might be considered a constituent of the oil, although actually it is a derived product, being the material removed by refining

the crude oil. Approximately a hundred million pounds of soapstock are produced annually from cottonseed oil in the United States alone. This material, which contains about 20 to 50% fatty acids together with concentrates of the minor non-glyceride constituents of the oil, is of interest in the use of cottonseed meal, since considerable quantities of the acidulated acids derived from soapstock are added back to solvent-extracted meals to improve appearance, physical properties, and palatability, and to add energy to the feed. (See Section VI.) Minor constituents of the soapstock may include phosphatides in quantities up to 12%, and gossypol and its derivatives in quantities ranging from less than 1 up to 10%, depending on processing conditions (42).

b. Protein

About 80% of the nitrogen in carefully defatted cottonseed (defatted with diethyl ether or petroleum ether without use of heat) is soluble in dilute sodium chloride solution; about 90% is soluble in dilute alkali; about 10 to 12% is soluble in water; and approximately 4 to 10% is non-protein nitrogen. The solubility of the nitrogen in cottonseed increases with increasing pH of the solvent; practically 100% is soluble at pH 12. There is also a maximum of solubility on the acid side, at a pH of about 1, but at this maximum only about 50% of the total nitrogen is soluble. The nitrogenous constituents are least soluble within the pH range of 3 to 6.

A large portion of the nitrogen which is extracted either with dilute salts or by alkali can be precipitated from solution by adjusting the pH of the solution to the range of minimum solubility, by addition of salts such as ammonium sulfate, or by dialysis. Protein preparations based on these principles were included among the classical researches on vegetable proteins by Ritthausen, Osborne and associates, and Jones and Csonka; this work has been mentioned briefly in Chapter 10 and is reviewed by Fontaine (43). On the basis of the classical nomenclature, as described in Chapters 2 and 3, the bulk of the proteins of cottonseed would be considered globulins; that is, proteins soluble in dilute salt solutions. Only a small portion of the proteins requires alkali for solubility (to be classified as glutelins), and another small portion of the proteins is water-soluble and might possibly be considered albumin. As was pointed out previously, this classification in many instances is artificial. Processing or heat treatment of cottonseed reduces the solubility of the proteins in salt solution and increases the amount of alkali needed for solution. This indicates the transitory nature of any classification of cottonseed proteins.

Cottonseed protein has been prepared, isolated, and evaluated for use in production of artificial fibers. In such a procedure the protein is extracted with either alkali or salt solution and is precipitated by adjusting the pH of the solution to that of minimum solubility. In one such preparation, as reported by Arthur (44), 85% of the nitrogen was extracted from solvent-extracted cottonseed by using a dilute alkali solution of pH 10. After clarification of the extract liquor, the protein was precipitated by adjusting the pH to 4 with sulfur dioxide. A yield of 30 to 35% by weight of protein, was obtained, corresponding to about 50 to 60% of the protein contained in the solvent-extracted meal.

The composition of various cottonseed protein preparations and fractions is given in Table V; the amino acid composition of cottonseed globulin is given in Table X along with the composition of protein in the meal. As can be seen from Table V, so-called cottonseed globulin with a nitrogen content of 18.2% can be prepared; some of the other fractions or preparations of the entire protein contain less nitrogen. This points to the limitations of the empirical factor 6.25 used to convert nitrogen content into crude protein, since some cottonseed protein fractions contain more than 16% nitrogen. A factor of 5.30 has been suggested by Jones (45) as being more accurate. For purposes of uniformity and for comparison with other meals, the factor of 6.25 is used here for cottonseed meal as it is used for other meals.

Most of the phosphorus in the protein preparations can be accounted for as phytin phosphorus; phytic acid and phytin are found in most oilseeds in combination with the proteins. Phytic acid can be removed from cottonseed protein by dialysis and by taking advantage of different effects of pH on the solubility of the phosphorus and nitrogen. These are clearly pointed out by Fontaine *et al.* (46), who give the percentage of nitrogen and phosphorus from cottonseed soluble at various pH values. Further discussion of phosphorus in cottonseed is found in a later section.

Regardless of methods of extraction and fractionation of cottonseed protein, all such preparations have been shown to be polydisperse materials when investigated in the ultracentrifuge or by electrophoresis (47). For other oilseeds, notably peanuts, evidence has been presented that the proteins are part of an association-dissociation system. Under certain conditions a unit of relatively small molecular weight can be observed, particularly in solvents containing urea. Whether this is also true for cottonseed proteins has not been determined.

About 10 to 12% of the nitrogen in cottonseed is extractable in water at the pH of minimum solubility, and about 20% of the nitrogen is not precipitated from alkaline extracts of cottonseed when the pH is adjusted to the range of minimum solubility. Much of this nitrogen is

TABLE V
NITROGEN, PHOSPHORUS, AND ASH CONTENT OF COTTONSEED PROTEIN ISOLATES

Method of preparation	Component				References
	Moisture	Ash	Nitrogen	Phosphorus	
	%	%	%	%	
1. Cottonseed was extracted with petroleum ether. Meal was extracted at pH 11, and extract was then acidified to pH 4.0. Protein was precipitated.	9.3	3.35	13.0	1.14	^a
2. Same as 1. After precipitation, protein was dialyzed for 72 hours.	10.6	0.22	14.0	1.16	^a
3. Cottonseed was extracted with commercial hexane in a pilot-plant batch extractor and then air-dried to remove solvent. Nitrogen was extracted at pH 10 and the protein precipitated at pH 4. It was washed with water and acetone and air-dried.	0	1.9 ^b	16.4 ^b	—	^c
4. Cottonseed was extracted with hexane to remove oil and then with butanone to remove gossypol. Nitrogen was extracted by water pH 6.5. The extract was clarified, dialyzed, and dried by lyophilization.	6.6	—	5.5 ^b	1.7 ^b	^d
5. Residue of 4 (after water extraction) was extracted with dilute sodium chloride solution. The extract was clarified, dialyzed, and dried by lyophilization.	2.3	—	17 ^b	0.35 ^b	^d
6. Residue of 5 (after water and salt extraction) was extracted with alkali at pH 10.5. Extract was clarified, dialyzed, and dried by lyophilization.	8.6	—	15.4 ^b	0.39 ^b	^d
7. α -Globulin	0	0.37 ^b	18.2 ^e	—	^f
8. β -Globulin	0	0.25 ^b	17.8 ^e	—	^f
9. Pentose protein	0	4.6 ^b	12.6 ^e	0.19 ^b	^f
10. Glutelin	0	7.35 ^b	15.3 ^e	0.35 ^b	^f
11. Globulin	0	1.57 ^b	18.1 ^b	0.50 ^b	^g

^a T. D. Fontaine, W. A. Pons, Jr., and G. W. Irving, Jr., *J. Biol. Chem.* **164**, 487 (1946).

^b Calculated on a moisture-free basis.

^c J. C. Arthur, Jr., and H. G. Many, *Textile Research J.* **19**, 605 (1949).

^d E. J. Conkerton, W. H. Martinez, G. E. Mann, and V. L. Frampton, *J. Agr. Food Chem.* **5**, 460 (1957).

^e Calculated on an ash- and moisture-free basis.

^f D. B. Jones and F. A. Csonka, *J. Biol. Chem.* **64**, 673 (1925).

^g J. C. Arthur, Jr., and B. H. Saik, *J. Colloid Sci.* **5**, 326 (1950).

non-protein nitrogen, that is, amino acids and small peptides as well as nitrogenous materials not regularly considered as constituents of proteins. Some of the protein is albumin; there are also allergens which are water-soluble and which are partly dialyzable. These have been studied extensively by Stevens and associates and have been reviewed by Fontaine (43). [See also Spies *et al.* (48).]

This description of the proteins of cottonseed refers primarily to the proteins as they exist in the seed or as they have been obtained from the seed by the gentlest of treatments, with a minimum of heat. This is a base line for the description of these proteins; in actual practice the proteins are modified considerably during processing, and their properties in the meal will differ from those in the seed.

c. Hulls

Hulls are composed primarily of crude fiber, approximately 33%, and non-nitrogenous constituents. There is little fat or protein in the hulls, and these might possibly have arisen from small amounts of kernels that could not be separated from the hull. The composition of hulls is approximately as follows: cellulose, 44%; pentosans, 29%; lignin, 22%; and ash 1.8%.

Hulls have their major use as constituents of cattle feeds. They also have applications as soil conditioners and in plastics. Furfural may be obtained from the pentosans by the same sort of a process that is employed in producing it from oat hulls. The yield from cottonseed hulls, however, is not quite so good as that from some other raw materials.

d. Linters

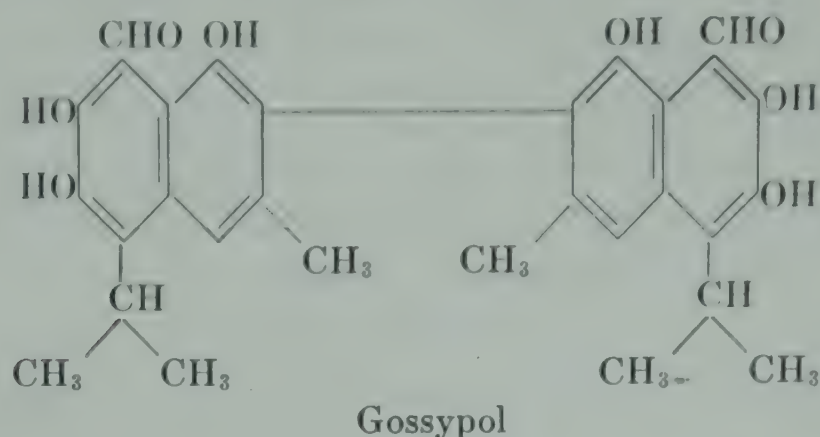
Linters are identical in appearance to cottonseed fibers but differ in length. They are composed primarily of cellulose (72 to 85%), with minor amounts of pectic substances, minerals, nitrogenous materials, waxes, resins, pigments, water-soluble carbohydrates, and acids. In the United States, linters are practically always removed before cottonseed is processed, and hence they are not of much consequence in cattle feeds. A large part of their use is as a source of cellulose.

3. Minor Constituents

a. Gossypol

Gossypol, so named by Marchlewski, is the major pigment of cottonseed. It is a yellow pigment and was once considered for use as a textile dye. Gossypol reacts as an aldehyde and as a polyphenol and, additionally, has fairly strong acid character. After an extensive series of

studies, Adams and co-workers (49) postulated the following structure for one of the tautomeric forms of gossypol:



Through the work of Shirley and Dean (50), who synthesized the hexamethyl ether of desapogossypol, and Edwards and Cashaw (51), who synthesized the hexamethyl ether of apogossypol, both known and described derivatives of gossypol itself, the "Adams" structure has been confirmed. The derivatives synthesized by these workers have properties identical with the corresponding compounds obtained from natural gossypol.

Boatner reviewed methods of preparing pure gossypol (29). It can be extracted from seed or meats with diethyl ether and then purified by precipitation as gossypol-acetic acid (52). It can be removed from meal with aniline as dianilinogossypol; King and Thurber (53) have described a procedure for recovering gossypol from this compound. Isolated pigment glands also can serve as a source of gossypol (54).

Gossypol is degraded by simple reactions to remove its aldehyde and also the isopropyl groups. It reacts with amines to form Schiff bases or anils. Along with these reactions are those which result from its polyphenol character. Under suitable conditions, gossypol will oxidize and polymerize to form quinones and colored bodies of higher molecular weight. These are the colored bodies that are probably in cottonseed oil and meal after processing.

Gossypol is an antioxidant and a polymerization inhibitor (55).

Aside from its chemical reactions, gossypol has physiological properties which influence the utilization of cottonseed meal for monogastric animals. For many years cottonseed meal was not used to any large extent for such animals because of the fear of toxic effects from gossypol. This fear has been dispelled in large part by the introduction of methods of analysis for gossypol (56-59) in cottonseed and demonstration that many commercial meals contain less than the amount which will produce toxic symptoms. (See Section VI.)

Gossypol, as it exists in a cottonseed kernel, is extracted entirely by aqueous acetone. A method has been developed, based on this property.

for the determination of so-called *free gossypol*. The meaning of free gossypol is an operational one; it is defined as the amount of gossypol that can be extracted from cottonseed or cottonseed meal with aqueous acetone. Gossypol is not determined directly but as the colored reaction product when mixed with aniline or *p*-anisidine. Compounds related to gossypol, such as gossypurpurin and diaminogossypol, give reaction products with identical color. In the course of processing, the amount of gossypol that is extracted with aqueous acetone decreases. Under conventional conditions of processing, gossypol which is rendered unextractable by aqueous acetone can be extracted if the meal is first heated with oxalic acid; this is the procedure used to determine *total gossypol*. That portion of the gossypol which cannot be extracted simply with aqueous acetone but is later extracted after acid hydrolysis is defined as *bound gossypol*; its actual significance is discussed later. From the known reactivity of gossypol and with the complex of materials with which it can react in the seed—proteins, free amino acids, carbohydrates, oil, phosphatides, etc.—there are undoubtedly many forms of so-called bound gossypol not necessarily related chemically to each other.

These two definitions of gossypol as it exists in the seed and meal have the practical significance that free gossypol is physiologically active; that is, it is toxic when fed in sufficient quantity to monogastric animals and is responsible for discoloration in eggs of hens fed cottonseed meal. Bound gossypol apparently does not have these physiological properties. For this reason these definitions have been a landmark in the history of extended use of cottonseed meal. Other methods for measuring gossypol in seed and meal have been proposed and used; among them are the colorimetric method of Lyman *et al.* (60), the antimony trichloride method of Boatner *et al.* (61, 62), and the phloroglucinol method of Storherr and Holley (63).

Although gossypol makes up about 50% of the weight of the pigment glands and occurs in the seed to the extent of 0.4 to 1.4% of the weight of the kernel, there is some question as to whether all the pigmentation and all the physiological effects attributed to gossypol account for the phenomena observed in the seed and its products. There is some contention that other materials in the pigment glands and seed have physiological properties (64). On this basis the physiological effect of pigment glands is not expected to be the same as that of pure gossypol isolated therefrom. Since little is known about the composition of pigment glands other than gossypol, this possibility, at the moment, is merely a field for conjecture and further experimentation.

Boatner (29, 65) isolated and named several other pigments re-

lated to gossypol. Gossypurpurin (66) is a dark purple, microcrystalline solid which melts at 200° to 204° and contains carbon, 63.15%; hydrogen, 6.15%; nitrogen 3.09%; and ash 2.86%; nitrogen is an intrinsic part of the molecule. Gossypurpurin can be prepared from gossypol by treatment with ammonia. It also is found in the seed where it increases in concentration during storage (67). It is said to be responsible for the dark pigmentation of glands in stored seed.

Other pigments mentioned by Boatner and others are gossycaerulin (68), a blue pigment; gossyfulvin, an orange-colored pigment (69, 70); and pigments unrelated to gossypol, such as anthocyanins and carotenoids.

b. Phosphorus Compounds

Most of the phosphorus in cottonseed, as in most other oilseeds, occurs in the form of phytic acid or phytin, its calcium, magnesium, and potassium salt. The amount will vary, depending on the condition of the seed, but will be of the order of magnitude of 75% of the total phosphorus. Phytic acid and its salts have influence on the solubility of the cottonseed proteins; their presence also has an influence on the utilization of phosphorus and calcium by animals fed cottonseed meal or other oilseed meals (46).

Stansbury *et al.* (71) reported on the influence of variety and environment on the phosphorus compounds in *G. hirsutum*. The total phosphorus content in cottonseed kernels (moisture- and oil-free basis) varied from 6.92 to 13.15 milligrams per gram, with a mean value of 10.5 milligrams per gram. Eight varieties were tested in thirteen locations throughout the United States. Changes in phosphorus content were influenced more by location than by variety, and the three-year mean for all varieties and locations showed that the distribution of phosphorus as phosphatide, inorganic, phytin, nucleic, and carbohydrate was, respectively, 6.8, 2.3, 76.6, 5.1, and 9.0% of the total phosphorus.

c. Carbohydrates

Total carbohydrate content of cottonseed on the basis of the weight of the dry kernels is approximately 13 to 15%. About 50% are mono-, di-, or trisaccharides; the others are hemicelluloses or pectin-like substances, and there is a small amount of cellulose. The principal component of the carbohydrate fraction is raffinose, a trisaccharide composed of fructose, glucose, and galactose (72). It has no reducing power but is converted by acid hydrolysis to its constituent monosaccharides and by dilute acid to fructose and melibiose. It is also

TABLE VI
VITAMINS IN COTTONSEED AND COTTONSEED MEAL

Vitamin	In seed	In meats	In meal				In flour	In special meals
	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
Thiamine	—	17.1–21.5 ^a	13.5	3.9	4.3–14.2 ^a	5.3	12.5	34–39.5
Riboflavin	2.3	—	9.0	5.5	—	5.3	4.1	—
Niacin	16	—	45	28.6	—	34	49	—
Pantothenic acid	11	—	14	9.7	14.0 ^b	10.3	19.2	—
Pyridoxine	—	—	—	—	—	—	9.8 ^c	—
Biotin	0.29	—	—	—	—	—	0.95	—
Inositol	3400	—	—	—	—	—	10,200	—
Folic acid	3.8	—	—	—	—	3.7	3.6	—
Carotene	—	—	—	—	—	0.22	—	—
α-Tocopherol	—	—	—	—	—	25	—	—
Choline, mg./g.	—	3.0 ^d	—	—	3.2–3.5 ^d	2.7	4.9 ^e	—
References	e		f	g		h	i	j

^a W. A. Pons, Jr., M. D. Murray, M. F. H. LeBlanc, Jr., and L. E. Castillon, *J. Am. Oil Chemists' Soc.* **30**, 130 (1953).

^b G. M. Briggs and F. S. Deft, in "The Vitamins" (W. H. Sebrell, Jr., and R. S. Harris, eds.), Vol. 2, p. 642. Academic Press, New York, 1954.

^c Reported as pyridoxine hydrochloride and choline chloride.

^d W. H. Griffith and J. F. Nyc, in "The Vitamins" (W. H. Sebrell, Jr., and R. S. Harris, eds.), Vol. 2, p. 60. Academic Press, New York, 1954.

^e V. H. Cheldelin and R. L. Lane, *Proc. Soc. Exptl. Biol. Med.* **54**, 53 (1943).

^f F. Hale and C. M. Lyman, in "Cottonseed and Cottonseed Products" (A. E. Bailey, ed.), p. 829. Interscience, New York, 1948.

^g E. C. Albritton, ed., "Standard Values in Nutrition and Metabolism," p. 138. Saunders, Philadelphia, 1954.

^h *Natl. Acad. Sci.—Natl. Research Council Publ.* **449** (1956). These are mean values for screw-pressed meals.

ⁱ Report from Traders Oil Mill, Fort Worth, Texas, 1953.

^j Butenone-extracted and gland-free meal prepared in pilot plant of Southern Regional Research Laboratory. A. M. Altschul, *Cotton Gin & Oil Mill Press* **52** (16), 24 (1951).

hydrolyzed enzymatically to yield either sucrose and galactose or melibiose and fructose. Raffinose occurs to the extent of 4 to 9% in cottonseed meal.

d. Vitamins

The content of vitamins in cottonseed and its products is given in Table VI. Cottonseed is a good source of thiamine. Since thiamine is relatively heat-labile, however, its content in cottonseed meal will vary

considerably, depending on the processing conditions. Cottonseed is a poor source of vitamin A. Cottonseed oil itself is a relatively good source of vitamin E, tocopherol; the amount found in the meal will depend on the amount of residual oil.

e. Minerals

The mineral content of cottonseed and cottonseed products is given in Table VII.

TABLE VII
MINERALS IN COTTONSEED AND COTTONSEED MEAL

Mineral	In seed	In kernels		In meal				In flour
	%	%	%	%	%	%	%	%
Calcium	0.18	0.19	—	0.33	0.18	0.18	0.19	0.38
Phosphorus	0.55	1.79	—	1.26	1.14	1.15	1.11	1.15
Sodium	0.14	0.71	—	0.20	—	0.03	0.04	—
Potassium	0.97	1.16	—	1.46	—	1.20	1.46	—
Magnesium	0.33	0.38	—	0.57	—	0.54	0.59	—
Iron	—	0.015	0.007	—	0.008	0.0097	—	0.016
Copper	—	0.0054	0.0015	—	0.0017	0.0019	—	—
Zinc	—	0.032	—	—	—	—	—	—
Manganese	—	0.0013	0.0013	—	0.0028	0.0023	—	—
Sulfur	—	—	—	—	—	0.40	0.41	—
References	a	b	c	a	d	e	f	g

^a J. D. Guthrie, C. L. Hoffpauir, M. F. Stansbury, and W. A. Reeves, *U. S. Dept. Agr., Bur. Agr. Ind. Chem. AIC-61* (Rev. March, 1949).

^b J. S. McHargue, *J. Am. Soc. Agron.* **18**, 1076 (1926).

^c D. C. Heinzelman and R. T. O'Connor, *J. Am. Oil Chemists' Soc.* **28**, 373 (1951).

^d E. C. Albritton, ed., "Standard Values in Nutrition and Metabolism," p. 138. Saunders, Philadelphia, 1954.

^e *Natl. Acad. Sci.—Natl. Research Council Publ.* **449** (1956).

^f *Yearbook Agr. U. S. Dept. Agr.* p. 532 (1939).

^g Report from Traders Oil Mill, Fort Worth, Texas, 1953.

4. Influence of Variety and Environment on Composition

Variety of seed and environment affect the relative amounts of oil, protein, and gossypol. Pope and Ware (73) concluded that oil, protein, and fuzz are all dependent on genetic constitution, and that consideration of these variables in a breeding program should result in isolation of lines superior in any one or all of these characteristics. Their data show that oil and protein contents are substantially independent as far as genetic constitution is concerned but are negatively associated when the effects of environment are considered.

This relationship is illustrated further by a study of eight varieties

grown at thirteen locations in the United States for three successive years. The various samples were analyzed for total gossypol, oil, and nitrogen content. Nitrogen in the kernels ranged from 4.75 to 7.34% with a mean of 6.31% (moisture-free basis); oil from 26.8 to 43.4% with a mean of 36.4%; and gossypol from 0.39 to 1.70% with a mean of 1.14%. Gossypol and oil contents were positively correlated, whereas there was a negative correlation between both of these and nitrogen content. Variety and location seemed to be about equal in their influence on the gossypol content of the kernel. Higher rainfall during the maturation period resulted in higher gossypol contents; higher mean temperature throughout the growing period produced the opposite effect (74-76). Neither of these two environmental factors had any significant effect on nitrogen content.

Boatner *et al.* (67) reported that Sea Island and Egyptian seed (*G. barbadense*) contained more gossypol and much more gossypurpurin than Upland seed (*G. hirsutum*). Gallup and Caskey (77-79) reported that from the time the bolls were mature and about to crack to the time they opened the gossypol content increased rapidly.

Storage of cottonseed results in a reduction of gossypol content and an increase in content of gossypurpurin (67, 80).

The existing knowledge would suggest that the preferred concentration of these materials can be influenced by selection of varieties and conditions of growth; indeed some research effort is being made in that direction. (See also Chapter 6.) Since cotton lint is the major economic product, it influences proportionately the goals of cotton-breeding programs. It might be expected, therefore, that attention will be given to influencing the yield of protein, oil, or gossypol only as it can be shown that this goal can be accomplished along with the best yields and quality of lint.

V. PROCESSING OF SEED AND COMPOSITION OF MEAL

1. Need for Considering the Two Together

Cottonseed meal cannot be considered a material of standard composition. Not only is it subject to the variations in composition of the seed itself, but it is also affected by the conditions of processing used to remove the oil. Hence the composition and usefulness of the meal are tied up with the conditions of processing. Variables in composition such as fiber and oil content (and hence protein content), condition of gossypol, content and availability of amino acids, content of vitamins, and quality of protein are among those which are influenced by conditions of processing.

Processing affects the use of cottonseed meal more for monogastric animals (non-ruminants) than for ruminants; therefore, for ruminants, which are the predominant feed market for cottonseed meal, conditions for processing are not quite so significant and can be controlled more easily by setting up standards such as are found in most trading rules. (See Section VI.)

It is because of the close relationship between processing conditions and composition that the two are treated together.

2. Methods of Processing

The description which follows is drawn largely from information about processing as it takes place in the United States. The principles of processing will hold equally as well in other parts of the world, although no doubt the details will differ. Moreover, there are areas where certain of the processing steps are omitted, as for example delinting and dehulling; although such changes do affect the fiber content of the meal, their effect on the oil, protein, and gossypol are secondary.

The procedures commonly employed in the United States for processing cottonseed are outlined in Table VIII.

Seed as it comes from the gin is contaminated, more or less, with trash such as stems, sticks, boll hulls, dirt, and pieces of metal. Cleaning is accomplished by means of rotating reels and shaker screens combined with pneumatic separators. After cleaning, the average Upland seed will contain (on a moisture-free basis) about 12 to 13% short fibers or linters, 32% hulls, and 55% meats or kernels. Short fibers are removed in delinting machines which are similar to the gins used in recovering lint cotton except that the saw blades are spaced at closer intervals. Some of the short fibers always remain with seeds, the amount depending on a number of factors such as quality of the seed, price of linters, and the purpose for which the seed is to be used. On an average, about 9% of linters are recovered. After the seeds are delinted, they are cracked usually with bar- or disk-type hullers. These machines are designed to crack or cut the seeds so that the meats fall away from the hulls without excessive crushing. Crushing at this stage would expell some of the oil which may then be absorbed by the hulls and consequently constitute a loss in oil yield. Meats are separated from hulls on shaker screens combined with pneumatic equipment. The extent of separation of the hulls from the meats will depend on a number of considerations, primarily the end use of the meal; these are discussed in a later section.

The general principles of oil extraction are described in Chapter 4. Cottonseed meats are either cracked or rolled to expose as much surface as possible to the moist hot vapors in the cookers and to the solvent used in extracting the oils. It is common practice in many oil mills to use a bank of rolls, five high and 60 inches long. The upper rolls are usually 16 inches and the lower ones 18 inches in diameter. Roll speed usually varies from 180 to 200 r.p.m.,

TABLE VIII
COTTONSEED PROCESSING—OUTLINE OF PROCESS

Seed cleaning	Trash removed, 4–8%
↓	
Delinting	Linters recovery, about 9%
↓	
Hulling	Hulls recovery, about 23%
↓	
Rolling	Flake thickness about 0.008 inch. Rolling is essential to effective cooking and oil removal. Converts part of free gossypol to bound gossypol.
↓	
Cooking	Aids in oil removal by increasing fluidity of oil, by partial coagulation of proteins and precipitation of phosphatides. Destroys molds and bacteria. Changes part of free gossypol to bound gossypol.
↓	
Oil recovery	
↓	

Type of extraction	Seed processed (United States) ^a				Residual oil ^b	Free gossypol content of meal ^b
	1945	1951	1952-53	1954-55		
	%	%	%	%	%	%
Hydraulic press	95	57	46	35	4-7	0.04-0.22
Screw press	5	31	33	35	3-5	0.03-0.08
Prepress, solvent extraction	—	6	21	30	Less than 1.0-3.0	0.02-0.06
Solvent extraction	—	<div> <div></div> <div></div> </div>				
Direct						
Direct, chemically treated					Less than 1.0-3.0	0.02-0.04

^a Taken in part from private communications and in part from L. V. Curtin, *Feedstuffs* **28** (4), 34 (1956).

^b Data consolidated from the following publications: L. V. Curtin, *Eastern Feed Merchant* **4** (11), 24 (1953); A. M. Altschul, *Natl. Cottonseed Products Assoc. Off. Proc.* **55**, 32 (1951); M. F. Stansbury, *Oil Mill Gaz.* **60** (4), 29 (1955); L. V. Curtin, *Feedstuffs* **28** (4), 34 (1956); W. A. Pons, Jr., F. H. Thurber, and C. L. Hoffpauir, *J. Am. Oil Chemists' Soc.* **32**, 98 (1955); W. A. Pons, Jr., M. D. Murray, M. F. H. LeBlanc, Jr., and L. E. Castillon, *J. Am. Oil Chemists' Soc.* **30**, 128 (1953); F. H. Thurber, H. L. E. Vix, W. A. Pons, Jr., A. J. Crovetto, and N. B. Knoepfler, *J. Am. Oil Chemists' Soc.* **31**, 384 (1954).

and the thickness of the flakes from about 0.008 to 0.035 inch. Water is generally added to the rolled meats prior to cooking to increase their moisture content to 12 to 17%. They are then stirred and heated in specially built, steam-heated cookers from 20 to 90 minutes, reaching maximum temperatures in the range of 200° to 270°F. During this cooking process the moisture content of the flakes is reduced to about 4 to 9%.

As late as 1945 approximately 95% of the cottonseed in the United States was processed with hydraulic presses. During the following ten years the change-over to equipment removing more oil and requiring less manpower was almost revolutionary. By 1954 approximately 35% of the oil produced in the United States was with hydraulic presses, recovery by screw pressing

had increased to almost 35%, and recovery with various types of solvent extraction had increased to about 30%.

The maximum pressure reached in hydraulic press equipment is about 2000 p.s.i. which is applied to the cooked meats for about 30 to 40 minutes, leaving a press cake with an oil content of about 4.5 to 7.5%. Pressures ranging up to about 20,000 p.s.i. are reached in the screw press, leaving a residual cake with an oil content ranging from about 2.5 to 5%. In prepress solvent-extraction plants, the screw presses are adjusted so that about two-thirds of the oil is pressed out mechanically. The press cake is then reflaked and extracted with petroleum solvent. By this procedure the oil in the extracted flakes is reduced to a range of about 0.4 to 1%. In direct solvent extraction plants, various types of extractors are used to remove the oil; these are described in Chapter 4. In one of the newer types of extraction (filtration-extraction) the flaked meats are cooked under carefully controlled conditions and the extracted meats are separated from the solvent-oil mixture by means of a horizontal rotary filter (81). In some direct solvent-extraction plants, the meats are not cooked before solvent extraction.

There is one modification of direct extraction which employs chemical treatment of the oil-free meal to reduce the free gossypol content to a level safe for feeding to monogastric animals. The meal is treated with organic amines which combine with the gossypol to form a non-toxic material which can be retained in the meal or removed by further extraction (82, 83). Pons and Hoffpauir have developed a modification of their analysis for free gossypol suitable for such meals (84).

3. Chemical Composition of Meal

a. Range in Composition

The range in composition of cottonseed meals is given in Table IX. Trading standards for meals in the United States are listed by the National Cottonseed Products Association (85). Typical meals would have the following characteristics:

Cottonseed meal, prime quality. Cottonseed meal, prime quality, must be finely ground, not necessarily bolted, must not have a sour or musty or burnt odor, must be free from excess of lint, and shall contain not less than 36 per cent of protein or 5.76 per cent of nitrogen. It shall be designated and sold according to its protein content.

Cottonseed meal with 36 per cent of protein or 5.76 per cent of nitrogen shall be termed "36 per cent protein cottonseed meal, prime quality," or "5.76 per cent nitrogen cottonseed meal, prime quality," and higher grades appropriately designated to reflect the guaranteed analysis.

There is another rule which defines degossypolized cottonseed meal; "so as to contain not more than 0.04 per cent free gossypol."

b. Amino Acids

The amino acid composition of the protein in cottonseed meal as well as of isolated cottonseed proteins is given in Table X. It would

TABLE IX
COTTONSEED MEALS—RANGE IN COMPOSITION

Origin	Process	Composition						References
		Moisture	Crude fat	Crude protein ^a	Carbohydrate			
					Nitrogen-free extract	Fiber	Ash	
United States	Hydraulic	% 7.6	% 5.1	% 41.2	% —	% —	% —	b
	Screw press	4.8	3.7	43.0	—	—	—	b
	Solvent extraction	7.6	0.8	42.4	—	—	—	b
	Prepress solvent extraction	—	0.1–2.3	39.6–45.7	—	—	—	c
India	All types	—	0.9 ^d	42.0 ^d	—	—	—	e
	Screw press	4.8–9.9	1–5	41.5	26.0	12.0	5.8	
		7.3 ^d	3.5–9.9	39.2–49.6	23.4–34.5	7.3–15.5	5.1–8.8	
			5.8 ^d	41.4 ^d	28.7 ^d	10.7 ^d	6.1 ^d	
		4.7–9.4	4.0–11.4	30–40	—	—	—	g
Brazil	Screw press	—	8–9	23–45	—	41–61 ^h	6–6.5	i
	Undecorticated	9.9	6.1	22	35	21	5.9	j
	Decorticated	7.5	10.0	35.4	32	7.7	7.4	j
	All types	—	1–7	25–44	—	—	—	k
Argentina	All types	7–10	1–7	38–42	—	—	—	l
Egypt	All types	—	2.5–10	13.5–27	—	—	—	m

^a Nitrogen $\times 6.25$.
^b "Average Report for the Season 1955–1956." Barrow-Agee Laboratories, Inc., Memphis, Tennessee.
^c W. A. Pons, Jr., F. H. Thurber, and C. L. Hoffpauir, *J. Am. Oil Chemists' Soc.* **32**, 102 (1955).
^d Mean value.
^e L. V. Curtin, *Feedstuffs* **28** (4), 34 (1956).
^f *Natl. Acad. Sci.—Nat. Research Council Publ.* **449** (1956).
^g B. L. Kulkarni, Osmania University, Hyderabad, India, private communication.
^h Total carbohydrate.
ⁱ K. C. Sen, *Indian Council of Agr. Research Bull.* **25**, 3 (1952).
^j A. Khan, *Oil and Oilseeds J. (India)* **4** (4), 17 (1951).
^k R. T. Kincey, Anderson Clayton & Cia, São Paulo, private communication.
^l R. Antonissen, Molinos Rio de la Plata, Buenos Aires, private communication.
^m N. S. Montassir, The Nile Ginning Co., Minia, Egypt, private communication.

TABLE X
AMINO ACID COMPOSITION OF COTTONSEED PROTEIN

Nitrogen, %	Meal		Flour		Globulin		Allergen
	—	—	—	—	—	18.0	12.1
Crude protein, ^a %	39.6	38.5	58.5	54.1	—	—	—
	g./16 g. of nitrogen				g./100 g. of protein		
Arginine	11.0	9.0	12.3	11.3	11.3	14.7	27.5
Histidine	2.7	2.5	2.5	2.6	3.0	3.4	3.4
Isoleucine	4.0	4.3	3.2	3.9	2.3	4.2	0.5
Leucine	6.2	6.0	5.8	6.2	5.5	7.1	1.0
Lysine	4.2	4.6	4.3	4.2	5.1	4.2	6.5
Methionine	1.5	0.96	0.68 ^b	1.6	3.0	—	0.6
Phenylalanine	5.2	5.2	5.8	5.2	7.5	8.1	1.0
Threonine	3.5	2.9	3.4	3.4	2.7	4.0	1.5
Tryptophan	1.6	1.3	1.2	1.6	1.3	—	0.6
Valine	5.0	4.6	5.0	4.8	5.8	6.1	0.8
Tyrosine	—	3.0	3.1	—	3.4	—	1.5
Aspartic acid	—	—	9.9	—	—	—	5.2
Glutamic acid	—	—	20.0	—	21.0	—	20.5
Proline	—	—	3.6	—	—	—	2.6
Glycine	—	—	4.4	—	—	—	3.0
Alanine	—	—	4.4	—	—	—	7.1
Serine	—	—	4.4	—	2.7	—	1.7
Cystine	—	—	—	—	1.0	—	3.1
References	c	d	e	c	f	g	h

^a Nitrogen × 6.25.
^b The authors raise a question about the accuracy of their value.
^c C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).
^d H. H. Williams, N. Y. (Cornell) *Agr. Expt. Sta. Mem.* **337**, 31 pp. (1955).
^e E. L. Andrianes and E. J. Bigwood, *Bull. soc. chim. biol.* **36** (4-5), 579 (1954).
^f T. D. Fontaine, in "Cottonseed and Cottonseed Products" (A. E. Bailey, ed.), p. 436. Interscience, New York, 1948.
^g G. R. Tristram, in "The Proteins" (H. Neurath and K. Bailey, eds.), Vol. I, Part A, p. 223. Academic Press, New York, 1953.
^h J. R. Spies, E. J. Coulson, D. C. Chambers, H. S. Bernton, H. Stevens, and J. H. Shimp, *J. Am. Chem. Soc.* **73**, 3998 (1951).

seem that cottonseed meal when fed with corn to monogastric animals is on the borderline of deficiency in lysine content, depending on the amount fed, other proteins in the diet, and the conditions of processing. Compared to soybean protein, cottonseed protein has less lysine but somewhat more methionine, and compared to peanut protein it has more of both lysine and methionine. This refers to total amino acids; availability as affected by processing will be discussed in a later section.

c. Vitamins and Minerals

The range in content of vitamins and minerals is given in Tables VI and VII; note the wide variation in thiamine content, ample evidence of its sensitivity to heat treatment during processing.

d. Carbohydrates

Cottonseed meal contains 5.3% total sugars, 13.2% pentosans and 9.6% cellulose (86).

e. Gossypol

The range of gossypol content of various types of meals is given in Tables VIII and IX.

4. Effect of Processing*a. Effect on Content of Fiber, Oil, and Protein*

(1) *Fiber*. The fiber content of cottonseed meal is a factor which can be controlled within wide limits by the extent of hulling and cleaning of the meals prior to the removal of the oil. At one extreme are the meals produced from undecorticated seeds which retain all the fiber of the original seed, concentrated by the removal of oil; at the other extreme is cottonseed flour which contains about 2% fiber and which is practically completely free of hull material. The crude protein content of meals in the United States is set by the trading rules at a minimum of 36%; this allows for partial removal of the hulls and yields materials with fiber contents ranging from 7 to 15%. Removal of hulls is tied in with the type of application intended for the meal. Meal intended for cattle feed may contain much more fiber, whereas meal intended for poultry or swine feeds and for human consumption must contain much less, and preferably a minimum amount. The technology for making a variety of meals is available; the type made depends on the economic incentive. As was pointed out in Chapters 6 and 10, manufacture of meals containing very little hull material or protein isolates accentuates the problem of disposal of the hulls which otherwise would have been incorporated in the meal. Solution of this problem is one of the factors in the economics of hull removal.

(2) *Oil*. The oil content of meals is a function of methods of processing, depending on the efficiency of the operation. Meals produced by hydraulic pressing contain the greatest amount, and those produced by solvent extraction the least, having contents of oil of less than 1% and often close to 0.5%. The technology for influencing the amount of

oil left in the meal is available; here again, the amount left in the cake is a question of economic consideration. It has been found that the complete removal of the oil, that is, to a level of 0.5% or so, results in a meal which is dusty, is less palatable, cannot be pelleted easily, and generally is not so acceptable for cattle feeding as are the meals containing 3 to 5% oil. It has become more and more the practice to add back to the meals fats of lesser economic value than the fat which has been removed. According to this practice, soapstock (or hydrolyzed vegetable fat which is crude acidulated soapstock) from the refining of cottonseed or soybean oil, or animal fats have been added back to meals to the point where improved appearance, palatability, and physical characteristics have been attained. (See Section VI.)

(3) *Protein*. The protein content of the meal is mostly a derived property, depending on the residual oil and fiber content. It can vary from about 22% in meal made from undecorticated seed to 60% in flour made from seed from which the hulls have been removed completely. There is also a genetic and environmental influence in protein content of the seed, as mentioned earlier.

b. Effects on Gossypol

Theories of rupture of pigment glands. Gossypol, as it exists in the seed, is contained in the pigment glands and, in such a condition, presumably does not react with the materials outside the gland. Thus, it is possible to account for the apparent stability in the seed of a reactive material such as gossypol within an environment containing carbohydrates, oil, and protein. There are, however, changes that take place during storage, even in the pigment glands themselves, that are apparent to the naked eye. For example, the color of pigment glands in fresh seed is a light yellow or tan; as seeds age or become damaged during storage, the color of the pigment glands changes to a dark purple and approaches black. This would indicate conversion of the gossypol into other pigments such as gossypurpurin or into quinones and polymerization products which have a more intense and darker color. There are also changes in the physical characteristics of the pigment glands. Pigment glands from fresh seeds are more easily ruptured by aqueous solvents than are those from older seeds. A sort of a tanning operation seems to take place which hardens the walls of the glands and makes them more difficult to rupture.

There are three principal means for rupture of pigment glands to facilitate the reaction of gossypol with the seed constituents: heat in the presence of moisture, the action of pressure and shear, and the action of solvents and chemical reagents. Merely by cooking in the

presence of moisture, 12 to 18%, it is possible to reduce considerably the amount of free gossypol in the seed (87, 88). If the cooking is done long enough and at a sufficiently high temperature it is possible to reduce the free gossypol almost completely. This latter is not a practical means for reducing free gossypol, since associated with this action is almost complete destruction of the protein value. Batson *et al.* (89) found that pressure with shear, as might take place in screw-press operation, ruptures pigment glands, whereas direct pressure, as in a hydraulic press, does not affect appreciably the pigment glands; therefore, the screw press, which involves shear and heat, is an efficient mechanism for rupture of pigment glands and binding of gossypol. The effect of various processing conditions on the properties of screw-pressed meals has been studied by Thurber *et al.*, who confirm that this process results in meals of low free gossypol content (90). The third means for rupture of pigment glands and removal of free gossypol is by use of solvents or by addition of chemicals. Extraction of the seed or meal with diethyl ether removes free gossypol. Dechary *et al.* (91) were able to reduce free gossypol content to a low level by extraction of oil-free meal with butanone (methyl ethyl ketone) containing 5 to 10% moisture. This method, although not a practical measure, has been employed in a pilot-plant operation to produce ton quantities of meal of low free-gossypol content with minimum heat treatment. This meal has been a standard in many feeding experiments with monogastric animals. And then there is the processing method mentioned previously which involves the treatment of cottonseed meal with aromatic amines such as aniline.

A combination of cooking and chemical treatment reported by King *et al.* (92) requires adjustment of the moisture content of the meal to approximately 30% and then reaction with dilute alkali. Under such conditions, the pigment glands are broken, gossypol is bound to the meal, and the resulting meal has a low free gossypol content. This is a cooking process which apparently accomplishes the purpose of reduction of free gossypol with much less damage to the protein than does ordinary cooking.

When processed under conditions promoting rupture of pigment glands and binding of gossypol, meals can be produced which have a free gossypol content ranging from 0.03 to 0.05%. This limit has been reached so often and by so many different means that it has aroused conjectures as to its meaning. There is one viewpoint that the remaining free gossypol is contained in the smallest and most refractory pigment glands which were not ruptured. Theoretically, therefore, it would be possible to reach "zero" free gossypol if complete rupture of the pigment glands were accomplished. Another point of view is that some of the materials which give rise to the color reactions of gossypol and are accounted as free gossypol in the analytical procedure are not gossypol and, therefore, cannot be bound.

c. Effect on Heat-Sensitive Components

Among the heat-sensitive components are some of the vitamins and the proteins.

(1) *Vitamins*. Thiamine is particularly sensitive to heat, and its content in cottonseed meal varies with the extent of heat treatment; indeed it was thought that thiamine content could be used as an indicator of heat damage. Ranges of thiamine content of cottonseed meal are from 4 to 15 micrograms per gram. (See Table VI.) No doubt some of the other B vitamins are affected by the heat treatment, but there are no data to indicate the ranges that are found.

(2) *Proteins*. The solubility of nitrogenous constituents in cottonseed meal is affected greatly by heat. Whereas practically 100% of the nitrogen in oil-free kernels is extracted by dilute alkali and strong acid, this amount can be reduced considerably by heat treatment. In addition to the changes in solubility of the nitrogen constituents, there are also changes in the nature of the proteins extracted from meals prepared at relatively low temperatures and from heat-damaged meals (93, 94). One change has been mentioned in Chapter 5; Fig. 6 of that chapter shows the changes in electrophoretic patterns of protein extracted from such meals. Conkerton *et al.* (95) demonstrated in another way the changes in the quality of the protein extracted from heated and unheated meal. From a meal containing low free gossypol and not subjected to heat damage, they were able to extract with salt a protein fraction containing 17% nitrogen. (See Table V.) When a portion of the same meal was autoclaved and then extracted in the same way, the amount extracted was 6% of the amount extracted from unautoclaved meal and the nitrogen content of the isolated material was very much less (4%), indicating that considerable non-protein material was bound to the protein.

The general reactions that take place when protein is heated have been described in Chapter 5. Protein can be damaged by heat when other material such as carbohydrate is present to react with it. In cottonseed there is the additional complicating factor of the presence of gossypol containing reactive aldehyde groups which can also bind with the protein and enter into some of these reactions.

Conkerton and associates (95) have also shown that when cottonseed meal is autoclaved under conditions which would damage the protein and reduce considerably its nutritive value to poultry, the content of lysine as determined by chemical methods is greatly reduced. Recently Martinez and Frampton (96) have shown that there is a variation in the lysine content of commercial cottonseed meals; values have been found ranging from 2.2 to 4.2%. Whether this means an actual

destruction of lysine or chemical alteration so that it cannot be detected by ion-exchange chromatography of hydrolyzates is not yet clear. Previous investigations of the lysine content of various cottonseed meals by use of microbiological assay of hydrolyzates have failed to reveal such differences.

Heat reduces the nutritive value of cottonseed meal to non-ruminants and also changes certain chemical properties. Attempts have been made to take advantage of these concomitant effects to develop a chemical measure of nutritive value which can be used to predict the effect of processing on the suitability of cottonseed meals for poultry and swine. Many measurements have been tried, including thiamine content, solubility in salt solutions, solubility in strong acid, and solubility in alkali. One of the more useful measurements has been that of nitrogen solubility in dilute alkali (97). This and other measurements are described in the next section.

VI. USES OF MEAL

1. For Ruminants

a. Nutritional Value and Feeding Practice

Cottonseed meal is one of the best of protein supplements for dairy cows, beef cattle, and sheep. Limitations which must be considered for effective utilization of this product in rations for swine and poultry either do not apply at all or are of minor significance for ruminating animals. For example, gossypol has no effect on cattle. Even whole raw cottonseed containing 1% or more of gossypol in the kernels can be fed safely to cattle day after day. Because the microorganisms of the ruminant can tear apart protein and rebuild it, the question of amino acid distribution in the protein need not be considered.

There have been times when cottonseed meal has been cheaper than grain. When this happens, the question to be considered is whether cottonseed meal can be substituted for grain as a source of energy after the minimum requirements for protein have been met. This problem emphasizes the fact that cottonseed meal has two important major nutritional values for ruminating animals: (1) as a source of protein and (2) as an energy source. When cottonseed meal is added to a ration deficient in protein for feeding beef cattle, each 100 pounds of the supplement will usually be equal in value to 250 to 300 pounds of corn or other grain (98). When cottonseed meal is fed beyond the amount needed to balance the ration, 100 pounds of the meal is worth only about as much as 96 pounds of corn (99) or a trifle less than 100 pounds of ground barley (100).

Neither cottonseed meal nor cottonseed hulls contains an appreciable amount of vitamin A or carotene. Fresh green grass is rich in carotene, but as the ranges dry up during the latter part of the summer the carotene rapidly disappears although much of the digestible carbohydrate remains. It is therefore imperative that some source of vitamin A be included in the ration when cottonseed meal is used as the protein concentrate with dried roughage; usually this is accomplished by feeding a small amount of bright green hay. In some instances stabilized vitamin A has been added to cottonseed pellets, but the practice has not been widespread, primarily owing to the cost of the stabilized vitamin.

TABLE XI
PRACTICE IN SUPPLEMENTATION OF BEEF CATTLE WITH COTTONSEED MEAL
ON DRY RANGE

Cattle	Meal lb./day
Dry cows	1½
Bred cows	2
Cows after calving	2½
Weaned calves and heifers	1-2
Bulls	2-3 ^a
Steers wintering on range	1-3 ^a

^a Depending on condition of range.

In the early days of feeding cottonseed meal to cattle in the United States it was believed that this meal caused blindness, stiffness of gait, swelling of the joints, and loss of appetite. Well-controlled investigations (101-105) showed that these symptoms were the result of vitamin A deficiency and had nothing to do with the characteristics of cottonseed meal except that it does not contain vitamin A.

(1) *Beef cattle.* The need for protein supplementation for cattle on the range increases as the season progresses and the range grasses dry up. The amount of cottonseed meal which should be fed varies with the condition and productive capacity of the range, and these factors are determined by the judgment of the experienced feeder. Table XI indicates the requirements of cattle on a more or less "average" dry range.

One of the major items of production cost is the labor required for the daily feeding of the protein supplement. A system for self-feeding based on the control of feed intake by adding salt to the meal has developed largely from the time of World War II when labor was particularly scarce. Under such a feeding system, a cow may consume from 1 to 2 pounds of salt daily, this amount being required to limit meal consumption to 2 pounds daily. The

system is not entirely automatic, since the amount of salt required to secure the desired consumption of protein concentrate varies with the condition of the range. It is obvious that a system such as this requires an abundant and readily available water supply.

In spite of vigorous criticism, largely on the basis of the load placed on the kidneys of the animals in order to excrete such large amounts of salt, the adoption of this feeding system continues to grow. The problem has been subjected to controlled experimentation. Savage and McIlvain (106) concluded that salt can be used successfully to control consumption of cottonseed meal self fed to Hereford steers on native ranges.

Riggs *et al.* (107) conducted experiments with cows and report that: "Salt may be used to limit the consumption of concentrates from self-fed mixtures. No ill effects from the use of these mixtures were observed when cows were allowed to become gradually adjusted to the high salt intake and were given access to an abundant supply of water at all times. Histological studies showed no kidney damage. Data from a digestion trial indicate a beneficial effect of high salt intake upon the digestibility of all the nutrients, particularly protein, crude fiber and nitrogen-free extract, with a less significant effect on ether extract digestion. High salt intake appeared to have no detrimental effect upon reproductive performance of the cows."

Cottonseed meal is one of the most widely used protein supplements for fattening cattle in the feed lot as well as for growing cattle on the range. The patterns for satisfactory rations are many and varied, depending on the age of the cattle and the availability of pasture and other feedstuffs. Since cottonseed hulls are available at the mills where the meals are produced, mixtures of 20% meal and 80% hulls are frequently sold. Such mixtures plus 2 to 3 pounds of green hay per day and a mineral supplement provide economical rations for fattening in 60 to 90 days. For higher finish, grain is added. Other typical rations are summarized in "Feeding Practices" (108) and by Ellis and Hodgson (108a).

Cottonseed meal does not have the laxative effect produced by linseed meal, and for this reason it is superior to linseed meal when added to cattle rations which are too laxative, such as those containing wet beet pulp or beet molasses (109). In more typical rations, cottonseed meal is worth somewhat less than linseed meal or soybean meal when each is fed as the only protein supplement (98). When cottonseed meal is combined with linseed meal, however, the value of the combination is about the same as that of linseed meal alone.

(2) *Dairy cattle.* Cottonseed meal ranks among the best of the protein supplements for dairy cattle. Some common rations for use under various conditions are shown in Table XII. When cottonseed meal is cheaper than grain, it can be used profitably to replace grain as an energy source. Milk production was slightly increased when the amount of cottonseed meal in the ration was increased from 11 to about 50%

TABLE XII
CONCENTRATE MIXTURES FOR DAIRY CATTLE^a

Feedstuff		Mixtures for producing cows (Type of roughage fed determines protein content needed in mixture.)															Calf start- er	Grow- ing mix- ture	Fresh- ening mix- ture
		20% protein mixtures to use with poor pasture and non-legume roughage (Choice of 5 mixtures; read down)					16% protein mixtures to use with fair pasture and mixed legume and non-legume roughage (Choice of 5 mixtures; read down)					14% protein mixtures to use with good pasture and ample legume roughage (Choice of 5 mixtures; read down)							
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13	No. 14	No. 15	No. 16	No. 17	No. 18
Cottonseed meal		lb. 716	lb. 740	lb. —	lb. 632	lb. 364	lb. 498	lb. 500	lb. —	lb. 384	lb. 420	lb. 376	lb. 376	lb. —	lb. 256	lb. 298	lb. 12	lb. 10	lb. 7
Corn or sorghum grain chops		—	—	—	—	636	—	—	—	788	820	—	—	—	—	942	24	63	25
Ear corn, ground, including husks		1084	—	—	—	—	1462	—	—	—	—	1584	—	—	—	—	—	—	—
Barley or wheat, rolled or ground		—	380	—	—	100	—	580	—	—	100	—	642	—	—	100	—	—	—
Oats, rolled or ground		—	—	—	584	400	—	—	—	788	300	—	—	—	852	300	37	24	25
Wheat bran or rice bran		—	—	—	—	200	—	—	—	—	120	—	—	—	—	120	12	—	20
Skim milk powder		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	12	—	—
Beet or citrus pulp or sweet potato meal		—	380	—	—	—	—	580	—	—	—	—	642	—	—	—	—	—	—
Molasses		—	100	—	—	100	—	100	—	—	100	—	100	—	—	100	—	—	10
Whole-pressed cottonseed (28% protein)		—	—	1150	—	—	—	200	776	—	—	—	200	572	—	—	—	—	—
Cottonseed hulls		—	200	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Alfalfa, ground		140	140	140	140	140	—	—	—	—	100	—	—	—	—	100	—	—	10
Calcium supplement		40	40	40	40	40	20	20	20	20	20	—	—	—	—	—	1	1	1
Bone meal		—	—	—	—	—	—	—	—	—	—	20	20	20	20	—	—	—	—
Salt (including trace minerals if needed)		20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	1	1	1
Total pounds in mixture		2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	100	100	100

^a From "Feeding Practices," Natl. Cottonseed Products Assoc. Bull. 32 (1955).

(110). The additional cottonseed meal, which replaced corn, oats, and wheat bran, was worth 10% more than the mixture of these feeds.

Before the rumen is fully functioning in young calves, they are susceptible to the harmful effects of gossypol. Hence, it is recommended that a concentrate for calves should not contain more than 20% of cottonseed meal (98). Holstein calves seem to be more susceptible than Jerseys (111).

Cottonseed meal has a tendency to produce butter fat of high melting point. Such butter has been characterized as having a gummy texture (112-115) and as being too hard or firm of body. Green pasture has just the opposite effect and produces butter which may be too soft

TABLE XIII
RATIONS FOR FATTENING LAMBS^a

Feedstuff	No. 1	No. 2	No. 3	No. 4	No. 5
	lb.	lb.	lb.	lb.	lb.
Ear corn or grain sorghum head chops	0	0	0	1	0
Corn, barley, wheat, or sorghum grain	1	1	$\frac{3}{4}$	0	1
Cottonseed meal or cake	$\frac{1}{5}$	$\frac{1}{3}$	$\frac{1}{7}$	$\frac{1}{4}$	$\frac{1}{3}$
Molasses	0	$\frac{1}{3}$	0	0	0
Dried beet pulp	0	0	$\frac{1}{2}$	0	0
Wet beet pulp	0	0	0	0	4
Alfalfa or other legume hay	$\frac{1}{2}$	0	$1\frac{3}{4}$	1	0
Cottonseed hulls	1	0	0	0	0
Sorghum fodder or non-legume hay	0	$1\frac{1}{2}$	0	$\frac{1}{2}$	$\frac{3}{4}$

^a From "Feeding Practices," *Natl. Cottonseed Products Assoc. Bull.* **32** (1955).

for satisfactory handling in warm climates. Feeding cottonseed meal in such cases was recommended for improving the keeping quality of butter (112); with modern refrigeration facilities this factor would seem to be of lesser significance.

Eckles and Palmer (112) found that the typical characteristics of "cottonseed meal" butter were produced by feeding cottonseed oil as well as the meal and that the effect of the meal could be accounted for on the basis of its oil content. In modern milling practice, much less oil is left in commercial cottonseed meal; hence the hardening effect on the butter is considerably less than it used to be.

(3) *Sheep*. Cottonseed meal is one of the most widely used protein supplements for sheep, both on the range and in the feedlot. In contrast with the results obtained with fattening cattle, cottonseed meal is essentially equal to linseed meal and soybean meal if considerable legume hay is fed and no more of the supplement is used than is actually neces-

sary (98). For comparative feeding trials with cottonseed meal and other protein supplements, the reader is referred to reports in the literature (116-120). Some typical ways in which cottonseed meal may be used in rations for fattening sheep are indicated in Table XIII.

b. Effect of Fat Content

Feeding trials designed to determine the effect of different types of processing on the value of cottonseed meal for ruminants have dealt largely with comparisons of hydraulic- and solvent-extracted meals. Solvent-extracted cottonseed meal usually contains 0.5 to 1.0% of fat as compared to about 5.0% or more in hydraulic-pressed meal. The question is, how important is it to keep this fat in the animal feed? A list of some of the more important functions of fat is given below.

1. Source of essential fatty acids
2. Source of energy
3. Improved digestion and absorption of other nutrients
4. Improved palatability
5. Improved physical characteristics of feed

Evaluation of the problem may be made in terms of these functions.

(1) *Source of essential fatty acids.* Most animals require small amounts of certain poly-unsaturated fatty acids. These fatty acids are constituents of grains, grasses, and other feeds as well as of cottonseed meal. The amount required by animals is so small that under practical conditions a deficiency of essential fatty acids is almost unheard of.

(2) *Source of energy.* That fat is a good food for animals with an energy equivalent 2.25 times that of carbohydrates is well established. When fat is removed and additional hulls are used to maintain the same level of protein content, there is a small but nevertheless a real difference in energy content of the feed.

(3) *Improved digestion and absorption of other nutrients.* It is well established that the absorption of the fat-soluble vitamins is poor in rations extremely low in fat. The absorption of calcium also seems to be affected. From a practical standpoint perhaps the effect on the absorption of carotene is of greatest importance. Experiments showing these effects (121) have employed wide differences in the fat content of the ration (2.5 to 6.5%).

When solvent-extracted meal with 1% fat is substituted for hydraulic-pressed meal with 6% fat, the differential is 5% in the meal; but if 10% of cottonseed meal is used in the ration, the fat differential is only 0.5%. The conclusion to be drawn is that such a change in the

fat content of the ration will not have any marked effect on carotene absorption. This conclusion has been substantiated by direct cattle-feeding trials (Riggs *et al.*, 122).

(4) *Improved palatability.* Small changes in the palatability of a product are difficult to measure. Both hydraulic-pressed and solvent-extracted cottonseed meals are well liked by cattle and sheep. Apparent differences in palatability would appear to be largely associated with differences in the physical state of the product.

(5) *Improved physical characteristics of feed.* Cottonseed meals of very low oil content such as solvent-extracted meals are dry and powdery. These meals are more difficult to handle in windy weather, losses due to sifting through bags are greater, and it is more difficult to maintain such meals as homogenous mixtures in mixed feeds. Dry powdery meals get in the nostrils of animals and tend to gum up in the mouths of birds. There is no question but that fat improves these qualities. From the standpoint of practical livestock production, it would appear that the effect of fat on the physical state of the meal is of greatest significance.

The results of practical feeding trials comparing hydraulic-pressed and solvent-extracted cottonseed meal are about as would be expected on the basis of the fundamental considerations discussed above. Pope *et al.* (123) reported essentially no difference in the two types of meal in wintering trials; but with fattening cattle, solvent-extracted meal as the protein supplement produced slightly less rapid gain than hydraulic-pressed meals. In feeding fattening cattle, energy relationships must be taken into consideration. Somewhat similar results were obtained in three other trials (124).

The unweighted average of the results of twenty-two different feeding trials comparing solvent-extracted and hydraulic-pressed cottonseed meal showed these to be practically the same (125). The average daily gain for cattle fed the solvent-extracted meal was 1.65 pounds, and for cattle fed hydraulic-pressed cottonseed meal it was 1.67 pounds. Although different types of tests were included, and this must be taken into consideration when interpreting such averages, these figures indicate that the differences in the nutritive value of the two types of meal for cattle must be very small.

c. Physical Forms of Meal and Cake

Cottonseed cake as it comes from the hydraulic-press oil mill is prepared for market in several different ways. It may be ground and sold as meal or cracked and screened into several different sizes. In other instances it is first ground and then pelleted into the desired sizes. Fre-

quently, cottonseed cake as it comes from the press is too hard to be eaten readily by the animals. The degree of hardness can be controlled by the mill operator, but to do so may result in slightly lowered efficiency in oil extraction. One of the chief advantages of pelleting is that hardness can be controlled more readily. The various sizes of cottonseed cake are described as follows:

1. Nut-size—cake which will pass through 1½-inch round perforation and over ¾-inch round perforation.
2. Sheep-size—cake which will pass through 7⁄8-inch round perforation and over 5⁄8-inch round perforation.
3. Pea-size—cake which will pass through 5⁄8-inch perforation and over 3⁄8-inch round perforation.
4. Pebble-size—a product consisting of fine particles and small pieces of cottonseed cake capable of passing through 3⁄8-inch round perforation.

Cottonseed pellets are prepared by extruding the material through a round hole. Cattle-size pellets are prepared with ¾- to 7⁄8-inch dies and sheep-size pellets with ½- to 5⁄8-inch dies. A pea-size pellet, 3⁄8 inch in diameter and ¾ to 1 inch long, is also available.

Square dies instead of round dies are used for the preparation of cottonseed cubes. The cattle-size measure 7⁄8 inch by 1¼ to 1½ inch and the sheep-size 5⁄8 inch by ½ to ¾ inch.

When cottonseed meal is fed under shelter with adequate feed troughs, meal, cake, or pellets can be used equally well. On the range, the protein supplement is frequently spread out on firm turf to be picked up from the ground by the cattle or sheep. In such instances, the ground form (meal) is entirely unsatisfactory because of loss on the ground.

The addition of 1 to 2% of hydrolyzed vegetable fat to solvent-extracted meal solves the pelleting problem and prevents dustiness in the material from any crushed pellets. At the same time the energy value of the fatty acids is added back to the meal. Specially designed machines are not required to pellet solvent meal to which hydrolyzed vegetable fat has been added. Hydrolyzed vegetable fat is added frequently to cottonseed meal even though it is not pelleted, for the purpose of obtaining improved physical characteristics, particularly to overcome dustiness.

2. For Poultry and Swine

a. History of Use

Years ago textbooks on animal husbandry frequently contained the statement that cottonseed meal is poisonous for hogs and should never be added to swine rations. This position held until 1930, when Hale (126) showed that cottonseed meal could be fed safely and profitably

to pigs provided that the amount was strictly limited to 9% of the ration. This conclusion was later verified by others (127, 128), and the way was opened for a limited expansion of cottonseed meal markets.

In 1930, cottonseed meal meant meal prepared by the hydraulic process. To date meals prepared by this process have always proved to be safe when fed as indicated above. In recent years, with the rapid expansion of solvent extraction as a process for manufacturing cottonseed meal, a few meals have appeared on the market with free gossypol content too high for safe use under the old recommendation. These recommendations were based on the safe level for feeding the most toxic meals then available on the market. With a better understanding of the nature of the toxicity, improved methods of measuring factors of toxicity and quality, and knowledge of the relationship between processing and properties of the meal, these recommendations have been largely supplanted. There now exist measures, none too perfect to be sure, to identify meals which can be fed in safety and which will be good sources of protein to non-ruminants.

The nature and properties of pigment glands were described in Section III; this was followed in Section IV with a description of gossypol and its derivatives, and a definition of the meaning of free and bound gossypol. It was shown that processing methods and conditions influence profoundly the amount and state of gossypol and the properties of the protein. In the pages to follow, this information will be applied in establishing a rational basis for feeding cottonseed meal to non-ruminants. A few of the high lights of historical interest are as follows:

In 1899, Marchlewski (129), a Polish chemist, purified a yellow colored pigment from cottonseed and named it gossypol.

In 1915, Withers and Carruth (130) isolated gossypol and showed that it has a poisonous effect similar to that of raw cottonseed when fed to experimental animals. These investigators and others (131) showed that gossypol has a detrimental effect on rabbits, rats, guinea pigs, and swine.

As early as 1913, Withers and Brewster (132) showed that the harmful effects of feeding cottonseed meal to experimental animals could be prevented by adding iron salts to the feed. This method of counteracting gossypol toxicity has been studied by numerous investigators (127, 133-136) since then. The practice of feeding iron salts for this purpose has never received general acceptance in practical animal production.

In 1917, Osborne and Mendel (137) reported that the toxicity of raw cottonseed was progressively reduced by steaming. Since that time the effect of moist heat treatment such as steaming and autoclaving (138-141) or cooking with water (137, 142) has been investigated further.

In 1928, Clark (143) showed that gossypol inactivated by moist heat treatment of the meal was not destroyed as was supposed at that time, but rather

was converted to an insoluble "bound" form. He postulated that this binding involved the free amino groups of cottonseed proteins.

Lyman *et al.* (88) showed that by the control of the processing factors, time, temperature, and moisture content, cottonseed meal which was safe for guinea pigs and swine, even when fed at high levels, could be manufactured in a typical hydraulic mill. Such meal consistently had a very low free gossypol content, but protein quality was disappointingly poor.

In November 1950, the first of a series of research conferences on cottonseed meal utilization, sponsored jointly by the Southern Regional Research Laboratory of the U. S. Department of Agriculture and the National Cottonseed Products Association, was held in New Orleans. Through this series of conferences in which investigators from various state agricultural experiment stations, industrial research organizations and the U. S. Department of Agriculture joined, an effective informal coordination of research efforts was accomplished.

It became apparent that there are two major and distinct divisions of the over-all problem. The first of these deals with the toxicity of gossypol, and the second has to do with protein quality as influenced by processing and other factors.

b. The Role of Gossypol

(1) *As a toxic agent.* Availability of pure gossypol prepared either from pigment glands or from oil or meal has made possible the study of its toxicity and an evaluation of its contribution to the physiological activity of cottonseed meal. Although the acute toxicity of gossypol is relatively low, when fed in small doses over a long period of time it is toxic and can cause death in animals. Eagle and associates and others studied the chronic toxicity of gossypol to dogs and reported its toxicity to rats, mice, chicks, and rabbits (144-148); the most sensitive animals apparently are guinea pigs, rabbits, and swine; next are dogs; and the least sensitive among those investigated are rats and poultry. In order to establish methods for manufacturing meal suitable for poultry and swine, it became imperative to know what amounts of free gossypol should be considered safe.

Heywang and Bird (149) fed New Hampshire and White Leghorn chicks diets containing ground raw decorticated cottonseed; or screw-pressed, hydraulic-pressed, solvent-extracted, or prepressed solvent-extracted cottonseed meals; or pure gossypol. The dietary levels of free gossypol furnished by these sources varied from about 0.008 to 0.075% in different experiments. Data on growth, diet consumption, and efficiency of diet utilization indicated that the free gossypol content in the diet should not be greater than 0.016% when fed to White Leghorn chicks or greater than 0.020% when fed to New Hampshire chicks.

In contrast, Couch *et al.* (150), with New Hampshire chicks and a somewhat different type of diet, found the tolerance level for free gossypol to be

0.06% of the diet. In these experiments pigment glands were used as a source of free gossypol. Previously Lillie and Bird (151) had reported that pigment glands and pure gossypol, administered daily by capsule at levels which supplied equivalent quantities of gossypol, were of approximately equal toxicity as judged by the effects on mortality and growth of chicks. These authors reported that gossypol administered by capsule inhibited the growth of New Hampshire chicks at a level of 0.063% of the diet.

The lack of agreement between investigators on the tolerance level of chicks for free gossypol was also characteristic of the informal preliminary reports on the tolerance level of swine for free gossypol. A large part of these discrepancies can now be explained on the basis of the discovery that tolerance levels for free gossypol vary with the amount of protein in the diet and also with the quality of the protein. Gallup and Reder (152) reported that gossypol toxicity was reduced by feeding high levels of protein in the diet, and this was also suggested by Clark (153). Clear-cut evidence for this principle was presented by Cabell and Earle (154), with rats as experimental animals. Hale and Lyman (155) studied the relationship of protein level in the diet to the gossypol tolerance level in pigs and found that 0.01% of the ration was the upper safe limit for free gossypol when the diet contained 15% protein. When the protein content of the ration was doubled, 0.03% free gossypol in the diet did not cause toxicity symptoms or lowered growth rate. These results are shown in Table XIV. The authors' interpretation of these findings is that gossypol tolerance levels for farm animals (non-ruminants) must be qualified to include a statement concerning the type and amount of protein in the diet.

Another point of view is that gossypol cannot account for all the toxic and growth-retarding effects obtained with cottonseed meal. As early as 1928, Gallup (156) suggested that other components of cooked cottonseed in addition to free and bound gossypol affected the nutritional value of the product. Eagle *et al.* (144) found that cottonseed glands added to diets of fasting rats, mice, guinea pigs, and rabbits were highly toxic, with large doses causing death, whereas an equivalent amount of pure gossypol affected the animals very little. In later gland fractionation studies these investigators (64) found that a water-acetone extract of the glands was more toxic than the original glands, but they did not report the separation and characterization of any substance other than gossypol and gossypol-related compounds, which have toxic properties. Gossypol combination products with proteins (157), carbohydrates (158), and glycine (159) have been prepared by Castillon and Altschul and others. They found that a freshly prepared gossypol-dextrose compound was more toxic for mice than an equivalent amount of pure gossypol and that a gossypol-glycine compound had no toxicity at all. Such experiments suggest that gossypol in the pigment glands may be in combination with substances which enhance its toxicity. See also Eagle and Davies (160).

Heywang *et al.* (161) mixed pure gossypol in a practical ration and after

144 hours were able to detect only about 20 to 25% of the added gossypol by chemical means. Feeding trials confirmed the conclusion that the gossypol had been destroyed or inactivated. Rapid destruction of gossypol added to feeds probably accounts for some of the apparent discrepancies in the early work on gossypol tolerance levels.

Thus far, non-toxic meals have always resulted from the reduction of the free gossypol content to low levels, regardless of how this reduction was accomplished. If indeed there are other important substances

TABLE XIV
EFFECT OF PROTEIN LEVEL IN THE RATION ON GOSSYPOL TOLERANCE IN GROWING FATTENING PIGS^a

	Standard protein level, 15% cottonseed protein % gossypol in diet				High protein level, 30% cottonseed protein % gossypol in diet			
	0	0.01	0.02	0.03	0	0.01	0.02	0.03
Ration	1	2	3	4	5	6	7	8
Number of animals ^b	8	8	8	8	8	8	8	8
Initial weight, lb.	58	58	58	58	58	58	58	58
Final weight, lb.	204	190	170 ^c	135 ^d	188	191	186	177
Average daily gain, lb.	1.74	1.57	1.31	1.16	1.55	1.58	1.52	1.42
Feed per 100-lb. gain, lb.	366	376	424	461	387	379	381	384
Number of deaths	None	None	2	4	None	None	None	None
Number of animals which lived, but showed gossy- pol toxicity symptoms	None	None	3	2	None	None	None	None

^a From F. Hale and C. M. Lyman, *J. Animal Sci.* **16**, 364 (1957); 84-day test (Dec. 28, 1955–Mar. 21, 1956).

^b Pigs fed in individual pens.

^c Six remaining animals.

^d Four remaining animals.

contributing to the toxicity of raw cottonseed, these must have similar solubilities, heat sensitivity, etc., to that of gossypol. Although the application of the concept of free gossypol has been of great practical benefit, the picture as it exists is not understood completely and other factors besides free gossypol might have physiological importance. These considerations in no way deter from the value of this concept but should introduce a degree of caution in considering it as the complete explanation of the physiologically important changes than can take place during processing.

The mechanism by which gossypol causes tissue damage is not fully understood. Accumulation of fluid in body cavities suggests that mem-

brane permeability may be involved. Gossypol induces hypoprothrombinemia in rabbits and pigs (162); and produces severe edema (163).

(2) *Bound gossypol and protein quality.* Effective utilization of cottonseed meal in rations for swine and poultry requires not only that the meal be low in free gossypol content but also that the quality of the protein be kept high. It has already been shown that cottonseed meals differ in composition, depending on conditions of processing. The nutritional value of cottonseed meals also varies over a wide range, and the statement is equally true for protein quality. Seeking a chemical test for evaluating protein quality in cottonseed meal, Lyman *et al.* (97) found a relationship between bound gossypol and protein quality; they included bound gossypol along with solubility measurements in a proposed chemical test. In tests (164) with prepressed solvent-extracted meals, all low in content of free gossypol, statistical analysis of the results reported by different laboratories showed highly significant negative correlations between total gossypol and chick growth rate, also between total gossypol and protein quality index with both rats and chicks. In meals all low in free gossypol, total gossypol is essentially equivalent to bound gossypol.

A relationship such as this might be expected on the basis of Clark's original concept of bound gossypol as gossypol which is tied up with protein through reaction with free amino groups on the protein molecules. Baliga and Lyman (165) devised a method for removing bound gossypol from samples of cottonseed meal without the application of heat or other drastic treatment. They found that the removal of bound gossypol from certain meals markedly improved protein quality as determined by protein repletion tests with rats and chick growth rate. The extent of the improvement is greatest with meals of high content of bound gossypol and low protein quality, provided that the meals have not been subjected to excessive heat damage by high-temperature processing (166). Changes in protein quality and chemical characteristics of a prepressed solvent-extracted meal on removal of bound gossypol are shown in Table XV. The same investigators formed a cottonseed protein-gossypol complex (without the application of heat) and showed that the nutritive value of the protein was reduced to less than half its original value. It was found that the only type of free amino group in cottonseed globulin is the lysine ϵ -amino groups and that on reaction with gossypol these free amino groups disappear (166).

Moist heat treatment can cause reactions between carbohydrates and proteins which are detrimental to proteins, and to a lesser extent moist heat treatment alone can do the same thing. It would certainly be a mistake to assume that bound gossypol is the only factor in connection

TABLE XV
EFFECT OF REMOVING BOUND GOSSYPOL ON THE NUTRITIONAL VALUE
OF COTTONSEED MEAL PROTEIN^a

Group	Meal description	Gossypol content		Nitrogen solubility in 0.02 N NaOH	Lysine availa- bility	Rat protein repletion value, weight increase in 10 days	Chick growth, average gain for 4 weeks
		Free	Bound				
		%	%	%	%	g.	g.
1	Original meal	0.033	1.32	61.0	54.9	26.0	121.7
2	Treated to remove free gossypol	0.003	1.10	69.0	54.1	29.0	157.8
3	Treated to remove bound gossypol	0.004	0.49 ^b	77.0	70.4	46.0	222.4
4	Standard meal (butanone extracted)	0.006	0.37	89.0	87.5	55.0	233.2

^a From B. P. Baliga and C. M. Lyman, *J. Am. Oil Chemists' Soc.* **34**, 23 (1957).

^b Includes 0.42% dianilino gossypol.

with protein quality in cottonseed meal. It appears, however, that it is one of the important factors.

c. Objective of Processing

Cottonseed meals of superior quality for rations for poultry and swine can be made by several different present-day commercial manufacturing methods, but control of processing variables is essential for the production of meals which are suitable. Based on the results of research during the past few years, the objectives should be:

1. To reduce free gossypol to low levels.
2. To maintain protein quality.
 - (a) By avoiding heat damage due to exposure to high temperatures.
 - (b) By keeping levels of protein-bound gossypol to the minimum.

The milling procedures which contribute to these objectives are briefly indicated below:

(1) *Low free gossypol*. An important factor is to ensure the breakage of the pigment glands; the theories of rupture of pigment glands have already been discussed. Adequate rolling of the kernels is the first

step. Moisture during cooking promotes gland breakage and also the inactivation of the gossypol in the meal (unfortunately this may occur at the expense of protein quality). Friction and pressure in a screw press is much more effective in breaking pigment glands than pressure alone as in the hydraulic press. In the direct solvent-extraction process, neither pressure nor friction is applied, and gland breakage depends on rolling and cooking.

In view of these considerations it might be predicted that screw-pressed and prepressed solvent-extracted meals are likely to be low in free gossypol; such is true. Hydraulic-pressed meals as a group are intermediate, and solvent-extracted meals are likely to be high in free gossypol unless special attention is given to reduction in free gossypol.

(2) *Protein quality*. For the maintenance of protein quality, minimum temperatures consistent with satisfactory oil extraction should be maintained in cooking and also in the screw press (if used). It appears that one of the difficulties in producing meals of high protein quality in a high-speed screw press is the elevated temperatures developed in the screw. As a group, direct solvent-extracted meals usually have high protein quality.

Although moist heat treatment is effective in reducing free gossypol, inactivation of gossypol in this way decidedly reduces protein quality. The answer is to accomplish the inactivation or removal of gossypol in some other way. One reason why the prepress solvent-extraction system is readily adaptable to the production of meals suitable for swine and poultry is that normally a considerable proportion of the gossypol is thrown into the oil in the pressing operation, to be removed later by refining.

An advantage of gossypol inactivation by reaction with a chemical compound is that the protein is left free. Additional practical milling procedures for gossypol inactivation are needed, particularly for solvent extraction.

Research contributions bearing on the points mentioned above are as follows:

Gastrock *et al.* (167) studied the preparation of meals and processing control needed for producing low free gossypol and for high protein solubility. Reuther *et al.* (168) investigated the effects of moisture content, rolling, and cooking on the chemical properties of hydraulic-pressed meals. The characteristics of prepressed solvent extracted meals were studied by Pons *et al.* (169). Information on gossypol material balance has been reported by Pons *et al.* (170). Haddon *et al.* (171) studied the chemical properties of cottonseed meals as related to processing conditions.

The importance of processing at low temperatures for production of meals of high nutritive value has been reported by Milligan and Bird (172). Tests

designed to give information on the mechanism of heat damage have been conducted by Condon *et al.* (173) and by Kuiken (174).

Reports on nutritional evaluation of meals made by different processes include the following: Altschul (a review) (175), Groschke *et al.* (176), Boatner *et al.* (177), German and Couch (178), Sure *et al.* (179), Morgan and Willimon (180), and Grau and Sweigart (181).

d. Chemical Properties of Suitable Meals

At the Third Conference on Cottonseed Processing as Related to Nutritive Value, held at New Orleans in November, 1953 (182), the following statements were passed as resolutions:

"Results presented thus far indicate that chick and broiler rations containing cottonseed meal and soybean meal in equal proportions on a nitrogen basis are equal or superior to rations based on either cottonseed meal or soybean meal alone, when the cottonseed meal has 0.04% or less of free gossypol and 75% or more of nitrogen solubility in 0.02 *N* NaOH solution."

"Preliminary indications are, insofar as free gossypol is concerned, that cottonseed meals having 0.04% or less of free gossypol can be fed in unrestricted proportion in balanced diets for chicks, broilers and swine."

These statements indicate that the free gossypol content should not be more than 0.04% of the meal. If 20% cottonseed meal were used in a broiler ration, this would be diluted to 0.008% free gossypol in the ration. Although investigators are not in agreement with respect to the exact tolerance level for chicks, this value falls below the lowest published figure (0.016% for White Leghorn chicks, Heywang and Bird, 149).

Less than 20% cottonseed meal is required to balance a corn-cottonseed meal ration for hogs. Hale and Lyman (155) found that 0.01% free gossypol in pig rations was safe in a 15% protein ration. Because gossypol tolerance levels vary with both the quantity and the quality of the protein in the ration, it is not possible to establish an exact figure for tolerance. It is quite probable that with protein of very poor quality 0.01% gossypol in the ration would be toxic for pigs. Meals with poor protein quality should not be used in swine rations.

All in all, the figure of 0.04% free gossypol as the upper limit for cottonseed meals to be used in both swine and poultry rations still seems to be valid.

A number of different possible chemical measures for protein quality were investigated. Thiamine content and nitrogen solubility in 0.5 *N* sodium chloride solution were tried and discarded. The test which so far has proved to be the most useful guide is nitrogen solubility in

0.02 *N* sodium hydroxide (Lyman *et al.*, 97). (The original suggestion for this test was made by Raymond Reiser.)

The value of this test for selecting meals of superior nutritive value is indicated by the results with prepressed solvent-extracted meals shown in Table XVI. The test will identify meals with high protein quality and with very low protein quality. In the intermediate range it cannot be relied on to distinguish between meals of similar but not

TABLE XVI
PROTEIN QUALITY AND CHEMICAL CHARACTERISTICS OF COMMERCIAL
COTTONSEED MEALS (UNITED STATES)^a

Mill designation	Number of samples	Chick growth rate index	Total gossypol	Nitrogen solubility in 0.02 <i>N</i> NaOH	Chemical index	Gossypol content of cottonseed kernels ^b
			%	%		%
D	2	75.2	0.72	81.6	96.0	0.65
H	3	74.6	0.84	77.6	91.3	0.74
I	3	74.6	0.73	77.0	90.6	0.67
E	3	69.5	0.85	74.1	87.2	0.67
J	3	71.8	0.81	70.0	82.4	0.69
A	1	52.1	0.86	68.5	79.7	0.69
F	3	64.3	1.05	69.2	65.9	0.81
B	3	59.9	1.06	67.9	64.1	0.91
C	2	54.6	1.02	65.8	64.5	0.76
G	3	51.8	1.23	68.8	55.9	1.01

^a From collaborative experiment reported by W. Y. Chang, J. R. Couch, and C. M. Lyman, *J. Am. Oil Chemists' Soc.* **32**, 106 (1955).

^b Rolled cottonseed meats used in the preparation of the meals.

quite equal nutritive value, particularly if these meals have been made by different processing methods.

Altschul (183) has discussed the limitations of nitrogen solubility as a measure of nutritive value, the principal one being that there is no theoretical reason for assuming that solubility per se has anything to do with nutritive value. Such a measurement, therefore, is empirical; the conditions which affect it may not always affect nutritive value in a similar manner. Thus, for any given process there is quite a good correlation between solubility and nutritive properties. But when a number of different processes are compared with each other, this relationship does not hold as well. Eagle (184) has challenged the value of analyses for both nitrogen solubility and free gossypol in predicting nutritive values of cottonseed meals.

It is hoped that even better chemical tests for protein quality will be developed. Until such time, nitrogen solubility in 0.02 *N* sodium hydroxide may serve as a practical guide in the production of superior quality meals for poultry and swine. A nitrogen solubility value of 75% as indicated in the 1953 conference resolution is ideal. Some mills have had difficulty in attaining this, and a value of 70% has been accepted rather widely.

At the Fourth Conference on Cottonseed Meal Processing as Related to Nutritional Value of the Meal, held in New Orleans in January, 1957, preliminary announcement of two new chemical procedures which may be of value in connection with protein quality evaluation were made; both were mentioned previously. One of these was the determination of lysine by the Moore and Stein column chromatography method (95, 96), and the other was the determination of the ϵ -amino groups of lysine (185, 186). The observation on lysine by Martinez and Frampton (96) may well serve as a basis for a method of chemical evaluation of nutritive value more directly related to conditions favoring nutrition, since it measures the amount of lysine in the meals. This approach will no doubt be tested thoroughly to determine whether it has usefulness in cottonseed. The small changes in lysine content, which might have much less effect in determining the nutritive value of material richer in lysine (such as soybean meal, for example), could easily be the critical factor in determining the nutritive value of cottonseed meal, particularly in rations with corn, where there is a stress on the need for lysine. The second measurement in which the free ϵ -amino groups of lysine are determined by reaction of the meal with 2,4-dinitrofluorobenzene may even prove more advantageous in elucidating the chemical changes in the protein during processing (185, 186).

e. Cottonseed Meal and the Storage Quality of Eggs

Eggs from hens fed cottonseed meal usually appear normal when first laid. If they are kept in cold storage for a month or longer, they develop a discoloration, particularly in the yolk. Although the nutritive value of such eggs is probably not impaired in any way, their appearance makes them unsalable. A typical description is as follows: The yolks may be olive green or almost black. In other cases the color is reddish. A mottled condition is common. A pink or almost red discoloration in the whites has been reported. The yolks are likely to be abnormally large and to have a thick gelatinous consistency.

Sherwood (187) reported that the discoloration was due to some substance associated with crude cottonseed oil. The investigations of Schaible *et al.* (188), Lorenz (189), and Swenson *et al.* (190) all show that the olive-green yolks are caused by gossypol in the feed. According to Lorenz (189), the gelatinous consistency of the yolks is also due to gossypol.

Lorenz and Almquist (191) presented evidence to show that the pink whites are due to some substance, not gossypol, which is characteristic of the malvaceous plants. They believed that this substance was identical to the substance in the oil which gives the Halphen color reaction.

A test for detecting eggs which would become discolored if stored was devised by Schaible *et al.* (192). The eggs are broken and allowed to stand in an atmosphere of ammonia. The yolks of the eggs which would have become discolored on storage turn to a chocolate brown in a very short time.

Feeding iron salts along with cottonseed meal affords at least a partial protection against egg discoloration. Iron salts have been shown to prevent the absorption of gossypol during digestion (190).

Although progress in recent years has made possible the commercial production of cottonseed meals of high protein quality which are entirely safe for poultry and swine, such meals will still cause the discoloration of eggs on storage. Heywang *et al.* (193) showed that as little as 0.001% free gossypol in the ration will cause some discoloration of eggs on storage. Heywang *et al.* (194) confirmed the reports of earlier investigators that there are two components of cottonseed which cause discoloration of eggs. One of these, as yet uncharacterized, causes pink albumin. It is suspected that the pink discoloration is caused by ingestion of a component of cottonseed oil.

An important fundamental contribution concerning the mechanism of egg yolk discoloration has been made by Grau and co-workers (195, 196). These authors showed that when gossypol is present in egg yolk it is not there in the free form but partly as a gossypol-cephalin complex. A procedure for the determination of gossypol-cephalin in fresh eggs is described. The results indicate that amino groups play an important part in gossypol metabolism in the laying hen.

Heywang *et al.* (197) found that pure gossypol had an adverse affect on egg hatchability when included in the diet of the hen at the 0.024% level. The report of Heywang and Bird (198) showed that 0.02% free gossypol in the diet adversely affected egg production and diet consumption.

An interesting approach to elimination of the problem of egg-yolk color was reported by Grau *et al.* (199). Treatment of cottonseed meal with phloroglucinol permits it to be fed to hens without resultant egg-yolk discoloration. Although the treatment is probably not practical, the result provides information about the nature of the problem.

Dechary *et al.* (200) found that some anils of gossypol such as those resulting from the reaction of gossypol with aniline or *p*-aminobenzoic acid were little different in their effect when fed to hens than gossypol itself. But anils of gossypol and normal aliphatic amines when fed to hens did not hurt the quality of the eggs. Moreover, these authors have evidence that suggests that the phenolic groups in gossypol are the ones responsible for the discoloration phenomenon.

The fact that the nature of the groups in gossypol responsible for the discoloration is better understood and that several derivatives have now been prepared which do not cause egg-yolk discoloration when fed to hens lends support to the feeling that this problem can be solved by a chemical approach.

3. For Man

Cottonseed flour was offered for sale in the United States as early as 1876. Fraps obtained samples of this flour which he referred to as cottonseed meal which had been finely ground and treated to remove the hulls as thoroughly as possible. The flour contained 48.25% protein, 12.16% fat, and 3.95% crude fiber; apparently it still contained some hull material (201). In the 1930's commercial production was begun of a flour, uniform in color, odor, and taste, low in fiber content, and high in content of protein. Originally this flour was made by hydraulic pressing. Carefully selected, well-filled mature seed, free from field, storage, and insect damage, was cleaned, delinted, and hulled by conventional procedures. All but traces of the hulls were then removed by screening and air separation. The meats were rolled and cooked for somewhat over an hour at 225°F. Time, temperature, and moisture conditions of cooking were adjusted to yield a light-colored product from which the oil could be removed by hydraulic pressing. The press cake was aged for about 30 days and then pulverized with a hammer mill, air separator system so that at least 97% passed through a 200-mesh screen. Flour thus prepared contained about 57 to 58% protein, 1 to 2% fiber, and 6% oil. Recovery per ton of cottonseed was approximately 300 pounds (202, 203).

The manufacture of cottonseed flour differs from that of meal in the following respects: (1) The seed is selected carefully. (2) Essentially all the hulls are removed before the meats are processed. (3) More oil is left in the press cake. (4) The press cake is pulverized and classified by means of a hammer mill, air separator system so that about 97% of the product passes through a 200-mesh screen. Tailings from the separator are not returned to the grinder, since the part of the press cake most readily pulverized produces the most desirable flour.

It is clear that the method of oil removal is not limited to any one procedure. Advantage may therefore be taken of the progress made in producing meals of high nutritive value and low free gossypol content.

Vitamin and mineral content and amino acid composition of cottonseed flour are given in Tables VI, VII, and X.

Little nutritional information is available on cottonseed flour *per se* compared to the extensive work reported on the meal. Moreover, most of that which has been published has been conducted on flour produced before the newer information on production of high-quality meals was available. There is really no essential difference between meal and flour when processed by advanced methods to reduce free gossypol and retain protein quality; primarily the difference lies in the fiber content. Hence the nutritional information already available on meal can, for

the most part, be applied to predicting what can be expected from a flour of similar quality.

As much as 10% of wheat flour used in bread can be replaced by cottonseed flour, and the resulting bread product will still retain the desirable appearance, flavor, texture, and eating qualities demanded by consumers. Because of color, only brown bread and other brown baked goods can be prepared with cottonseed flour replacing some of the wheat flour. Some of the formulas used in making the baked goods are reported by Summers *et al.* (204). Breads baked according to these formulas were tested in nutrition studies on rats. The addition of cottonseed flour to wheat flour (10 parts to 100 parts) in bread making increased both the quantity and quality of the protein. Ten grams of cottonseed flour was about as efficient as 4 grams of milk solids in increasing the protein quality of the bread (205).

Overman reported that both cottonseed and soybean flour were effective in delaying the development of organoleptic rancidity in raw food mixes and to a lesser extent in baked pastry (206).

Spies and associates described the allergens of cottonseed. The same procedures developed for isolating the allergenic fractions were used in isolating similar proteoses from other oilseeds and nuts. Yields and composition of these allergen preparations and the results of immunological tests are given by them (48).

There is no clear-cut information on the frequency of allergy to cottonseed protein as compared to other protein sources. It is generally felt that allergy to cottonseed protein is no more and in some cases less frequent than allergy to common protein sources such as wheat, egg, and milk. Many people in the United States and Canada consume cottonseed flour daily. It is often an ingredient of biscuits, crackers, doughnuts, and prepared food mixes. Any extraordinary frequency of allergic response would have been noted. Bread, doughnuts, and cakes containing cottonseed flour were sold in the Oklahoma A. & M. College food store and cafeteria for approximately 18 months as part of the study previously reported. No evidence of allergenic or other disturbances was reported; foods with and without cottonseed flour were equally acceptable (204).

VII. TRENDS IN PRODUCTION AND UTILIZATION OF COTTONSEED MEAL

Economics of cotton lint production will continue to dominate the cottonseed picture. Cottonseed is truly a by-product of cotton production. Even though its contribution to the value of the crop is substantial, its influence on trends in production or breeding will be secondary.

Cottonseed meal will continue to become more of a factor in the income from cottonseed. Particularly as the meal is used more in higher-priced markets, its economic contribution will approach that of the oil and may in some instances become greater. Hence, processing of cottonseed will take more account of meal quality, and there will be greater effort to produce meals which can compete in higher-priced markets.

Competition of urea and the like and the possibilities for use of cottonseed meal in markets for non-ruminants will probably diminish the percentage of meal given to cattle. More cottonseed meals are now available for poultry rations. Some of these can be added to corn-protein rations as a main source of protein supplement, but most of the cottonseed meals serve to the greatest advantage when blended with a better source of lysine such as soybean meal. In the United States the presence of vast amounts of soybean meal is a fortunate circumstance which will enhance the chance of more cottonseed meal's going into poultry rations; these chances will increase as the quantity of protein in the meals is increased to 50% or so concomitant with elimination of gossypol and improvement in quality.

A cottonseed flour is already in manufacture and in use in the United States for incorporation in human foods. Cottonseed flour as a protein supplement in human foods is being investigated in various parts of the world where cottonseed flour could be made available for such purpose. A logical extension of this approach would be to test cottonseed protein isolate in human foods.

By far the most potent factor affecting trends in the economics and application of cottonseed meal is the effect of research. It has been mentioned previously that in the United States there has been an active, coordinated research program on cottonseed meal involving the National Cottonseed Products Association, the Southern Regional Research Laboratory of the United States Department of Agriculture in New Orleans, State Agricultural Experiment Stations in the cotton-growing states,* laboratories of some of the cottonseed processing and feed-mixing firms, and research laboratories of the United States Department of Agriculture in Beltsville, Maryland. Exchange of views and informal planning for the future have taken place through the medium of four public research conferences held at the Southern Regional Research Laboratory, the last of which was held January 14-16, 1957. Out of these conferences have come statements, mentioned earlier, which rep-

* These include, principally, Arizona, Arkansas, California, North and South Carolina, Florida, Georgia, Louisiana, Mississippi, Oklahoma, and Texas, although others have been involved from time to time.

resent the best informed consensus on the current use of cottonseed meal. These statements have influenced processors and feeders and encouraged them to use cottonseed meal in higher quality markets. As a result of this effort, cottonseed meal in the United States has found substantial use in non-ruminant rations. Many of the publications that arose out of this cooperative program are summarized in a bibliography by Rubins *et al.* (207).

Although considerably more research progress is needed for utilization of cottonseed meal to the optimum advantage, there is reason to believe that the research program of the magnitude that is underway may result in the solution of some of the problems. There is reason to believe that methods will be developed which will provide meals that can be fed to laying hens without fear of egg-yolk discoloration; that cottonseed meals will be fed to swine with complete confidence; that it will be possible to produce economically cottonseed meals with minimum protein damage; and that a way will be found to measure heat damage so as to provide a chemical basis for control of production and use of the meal. Although major attention has been on gossypol as affecting the quality of the meal, there are no doubt other materials in the seed and meal which influence its use as a foodstuff. These constitute the challenge and the opportunity of the future.

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CHAPTER 18

SESAME MEAL

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I. INTRODUCTION

Sesame is one of the oldest vegetable oil crops cultivated by man; the seed, oil, and meal have served as a staple food in the dietary regimen of Asian peoples for generations. Its antiquity is documented by many ancient literary references. Herodotus describes the field-scale culture of sesame as early as 450 B.C. Citations on sesame appear in old Hebrew and Egyptian scripts, and "Marco Polo's Travels" contains references to the production of sesame oil in Persia and other countries of the Far East.

Sesame has been cultivated in Africa, throughout Asia Minor, India, China, Manchuria, Japan, and in parts of Europe for centuries. It is a new oilseed crop in the Western Hemisphere, production having been initiated during the early 1930's in South America, Central America, and Mexico. Originally, sesame was introduced and grown in the United States in the late seventeenth century by Negro slaves, who brought the seed from West Africa to the plantations along the South Carolina coast. The development of improved indehiscent strains of sesame has provided the stimulus and potential for large-scale production in the Cotton Belt areas.

Prior to World War II, the main uses of sesame seed in the Western World were limited to oil extraction, as a topping for bakery products, and the manufacture of confectionary specialties. The critical shortages of protein concentrates during and immediately after World War II stimulated interest in the use of sesame meal in poultry and livestock rations. Relatively small quantities of sesame meal from Mexican and Central American processors are currently imported by Pacific Coast feed manufacturers for use in poultry rations. The bulk of the sesame meal produced in the majority of countries is reserved for the feeding of domestic livestock.

Owing to the dehiscent nature of the sesame capsule, the harvesting

of sesame in the Eastern Hemisphere is mainly by hand, as mechanical harvesting methods are not readily adapted or employed. It was apparent to agronomic experts that large-scale production of sesame could be feasible only if the crop were adapted to mechanical harvesting. This basic requirement posed a challenge to the plant geneticists and agronomists. The realization of this goal was "triggered" by the discovery of the first mutant "closed-pod, indehiscent-type" nonshattering sesame in 1943 by Langham (1) in Venezuela. This important finding fostered the sesame breeding programs in Venezuela and the United States and opened the door for the development of varieties of sesame that are applicable to mechanical harvesting on a commercial basis.

Several varieties of sesame have been planted and harvested by farmers in the South and Southwest areas of the United States with encouraging results. Many Texas counties have reported average yields of 700 pounds of sesame seed per acre. Sesame has the potential for the development as a major oilseed crop in the United States and other areas of the Western Hemisphere. Its high yield of oil per acre, the stability of the processed oil, the excellent protein quality of the meal—these are the attributes of sesame that are looked on favorably by oilseed processors.

In spite of the importance of sesame to the agricultural economy of many countries, there has not been sufficient research effort on sesame seed, oil, and meal. This will be all the more apparent as we note gaps in our knowledge which limit full effective utilization of sesame and its products. The valuable contributions to the improvement of sesame by many research workers are appreciated, although their individual papers are not all recognized by appropriate references in the text.

II. PRODUCTION AND TRADE

1. World-Wide

During the season of July 1953–June 1954, approximately 13.5 million acres were planted to sesame throughout the world with an estimated sesame seed production of 1.9 million long tons (2). Tables I and II list the figures for acreage and sesame seed production for various countries during the growing seasons of 1951, 1952, and 1953. China, Manchuria, and India are the world's largest producers of sesame, accounting for approximately 70% of the world crop. Other important producers are Sudan, Burma, Turkey, and Mexico.

In spite of the primitive cultural practices associated with the production of sesame in many countries, and the tendency of some governments to encourage the growing of competitive oilseed crops of higher yields, the level of world production of sesame is being maintained.

World trade in sesame seed accounted for about 9% of the 1953 production. The sharp increase in exports of seed in 1953 over previous years was due to increased shipments of seed from China to Western Europe. Western Germany imported 33,600 tons of seed in 1953, and the United Kingdom 19,000 tons out of an available world import

TABLE I
WORLD AREAS UNDER SESAME CULTURE^a

Country	1951	1952	1953
	or 1951-52	or 1952-53	or 1953-54
Thousands of acres			
Cyprus	2	4	3
India	5945	5860	6132
Nigeria	110	120	130
Pakistan	198	206	216
Tanganyika	31	49	—
Uganda	198	211	212
Anglo-Egyptian Sudan	170	340	(300) ^b
Burma	1332	1328	1352
China and Manchuria	(3500) ^b	(3500) ^b	(3500) ^b
Egypt	35	43	40
El Salvador	12	11	(8) ^b
Greece	87	89	90
Iraq	33	51	—
Japan	21	23	24
Mexico	420	420	371
Nicaragua	50	41	26
Thailand	45	42	37
Turkey	146	139	174
Others	790	795	810
	13,125	13,272	13,425

^a Data from U.K. Commonwealth Economic Committee, "Vegetable Oils and Oilseeds," H. M. Stationery Office, London, 1954.

^b Figures in parentheses are approximate unofficial estimates.

supply of 197,000 tons. Nicaragua and El Salvador raise sesame mainly for export; in the majority of countries practically the entire harvest is reserved for local use. Only small quantities of sesame oil and meal are traded in the world market.

2. United States

Sesame has not been cultivated extensively in the United States; however, access to improved strains of sesame which are adapted to

mechanical harvesting heralds the possible inauguration of a new cash crop for the Cotton Belt areas. Commercial plantings in Texas were started in 1953, and 420,000 pounds of sesame seed was harvested. The 1954 production amounted to 1.2 million pounds, and the annual crop is

TABLE II
WORLD PRODUCTION OF SESAME SEED^a

Country	1951	1952	1953
	or 1951-52	or 1952-53	or 1953-54
Thousands of long tons			
India	445	460	531
Nigeria	12	14	14
Pakistan	34	36	36
Tanganyika	5	8	—
Uganda	26	29	30
Anglo-Egyptian Sudan	40	130	(130) ^b
Burma	48	54	44
China and Manchuria	(785) ^b	(760) ^b	(820) ^b
Colombia	12	(10) ^b	(8) ^b
Egypt	12	14	13
El Salvador	4	3	2
Formosa	2	(2) ^b	(2) ^b
Greece	11	8	16
Iraq	9	12	—
Japan	6	6	5
Mexico	85	89	74
Nicaragua	15	14	5
Thailand	7	9	8
Turkey	28	29	37
Venezuela	2	2	7
Others	131	137	132
Total	1719	1826	1914

^a Data from U.K. Commonwealth Economic Committee, "Vegetable Oils and Oilseeds," p. 51, H. M. Stationery Office, London, 1954. See also: *U.S. Dept. Agr., Foreign Agr. Circ. FFO 13-57* (1957).

^b Figures in parentheses are approximate unofficial estimates.

expected to exceed 5 million pounds. Approximately half of the 1954 crop was sold to bakers, the balance exported to foreign oilseed processors. Since the baking industry can use between 1 and 2 million pounds of seed annually, it is obvious that the future market for sesame will soon be overtaxed unless large-scale crushing operations are instituted.

3. Yields

It is difficult to determine a world-wide average yield of sesame seed per acre with any degree of accuracy. By employing the hazardous conclusion that all acreage planted to sesame in 1953 was harvested, a calculated yield of 320 pounds of seed per acre is obtained. There are wide variations in seed yields from various countries as well as among sesame-growing areas within a country. Sikka* reported yields in 1952-53 for fourteen states in India of 64 to 517 pounds per acre. Kinman and Stark (3) published mean yields of seed, oil, and protein for seven varieties grown at eight locations in the United States of 662, 344, and 163 pounds per acre, respectively. Although the yield of sesame per acre is lower than many competitive oilseed crops, the average yield of oil per acre is considerably greater. Under average conditions of growing sesame, yields of 700 pounds of seed per acre are not unusual, and the probability of higher yields is possible.

III. BOTANICAL AND BREEDING INFORMATION

1. Botanical Data

Sesame, *Sesamum indicum* L., belongs to the Pedaliaceae family which consists of sixteen genera and sixty species generally found in tropical and subtropical areas. Because of the wide distribution of the genus *Sesamum* throughout the world, it is difficult to assign a specific region as the primary center of origin. The preponderance of evidence appears to favor Afghanistan or India. Seventeen species have been reported as found in Africa, and two wild species have been located in India (4). It is not known definitely whether any of these species other than *S. indicum* are cultivated.

Sesamum indicum is an erect, annual plant varying in height from approximately 2 to 6 feet at maturity. It matures in 70 to 150 days, depending on the strain and environmental conditions. According to Ram and Row (5), late-maturing types have a deeper tap root and more extensive secondary and tertiary roots than early-maturing types. The leaves of the sesame plant can be described as dentate, palmate, and entire. One to four trumpet-shaped flowers are borne in each leaf axil with corollas varying in color from white to purple (6). The fruit of the sesame plant consists of a bi-, tri-, or tetralocular pod, elliptical in shape. Each locule (pod chamber) is divided by a septum into two parallel cells containing from fifteen to twenty seeds each (Fig. 1).

* S. M. Sikka, personal communication, 1955.

Seed coat color may vary from white to black, depending on the variety; however, the chemical composition of the seed shows little variation.

2. Breeding Improved Varieties

The trend in breeding improved strains of sesame, particularly in the Western Hemisphere, is toward the development of suitable indehiscent (nonshattering) varieties that can be harvested profitably by mechanical means. Qualities sought after by sesame breeders in developing improved strains for a given set of growing and soil conditions are (1) uniform ripening of seed pods from base of plant to the top; (2) high seed yields; (3) maximum resistance to insects and disease

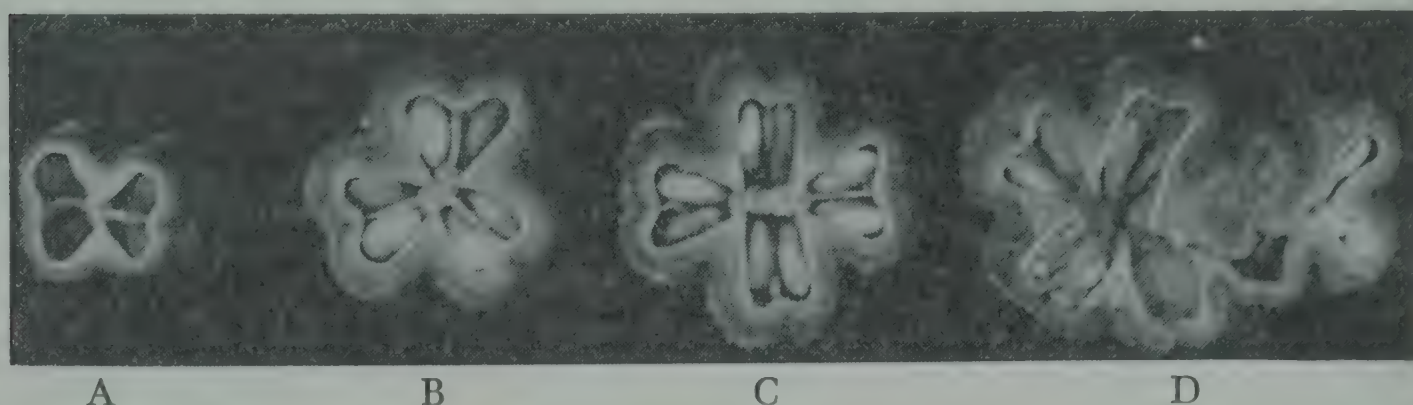


FIG. 1. Cross section of sesame pods showing the following types: A, bilocular; B, trilocular; C, tetralocular; D, abnormal type with more than four locules. [From E. H. Collister, *Texas Research Foundation Bull.* 4, 8 (1955).]

(4) adaptability to complete mechanical harvesting. Breeding methods commonly employed in developing dehiscent and indehiscent strains of sesame are use of pedigree, backcross, and multiple cross.

Breeding and improvement studies on sesame were pioneered in the United States at the South Carolina Experiment Station in 1943 by utilizing dehiscent strains. The main disadvantage of dehiscent varieties of sesame, profuse shattering of the seeds at maturity, was attacked vigorously by sesame breeders in 1948 when the indehiscent strain of sesame discovered by Langham (1) in Venezuela was introduced to research workers in the United States. Availability of this strain, along with the access to seed from a large number of species of *Sesamum*, accelerated breeding research in the United States. Following the lead of the workers at the South Carolina Experiment Station, sesame breeding programs were inaugurated at the University of Nebraska, the Texas Agricultural Experiment Station, and the Texas Research Foundation. Martin (7) has shown that under proper greenhouse conditions three generations of sesame per year may be grown, resulting in a definite speed-up in breeding techniques. As a result of the contributions of the sesame breeders, three types of sesame are available. They may be classed as dehiscent, semi-dehiscent, and indehiscent. Figure 2 illustrates the prolific seed production of an indehiscent strain, P-15, developed at the Texas Research Foundation, Renner, Texas. This experimental strain has excellent threshing characteristics and produced 860 pounds per acre of seed containing 28% protein and 51%



FIG. 2. Close-up of P-15 at maturity, showing the prolific seed production under droughty growing conditions. [From E. H. Collister, *Texas Research Foundation Bull.* 4, 23 (1955).]

oil. Renner No. 1, an improved dehiscent strain of sesame, was released by the Texas Research Foundation in 1953 and distributed to farmers in 1954. Renner No. 2, an improved dehiscent strain of high productivity, is under evaluation at Renner. The new indehiscent variety, Rio, developed at the Texas Agricultural Experiment Station, is also undergoing tests in experimental fields in Texas and other areas. Palmetto, a new indehiscent variety, was released in 1955 by the South Carolina Agricultural Experiment Station. Palmetto sesame seed contains about 28% protein and 48% oil, with yields ranging from 300 to 1000 pounds of seed per acre. Results of sesame breeding experiments appear in papers by Martin (8), Hoffman and Claassen (9), Kinman (10), Collier (11), Parthasarathy and Kedarnath (4), Langham and Rodriguez (12), Cano (13), and Kinman and Martin (14).

IV. GROWING AND HARVESTING

1. Growing

a. Adaptation and Cultural Requirements

Sesame is a warm-weather and short-season crop (70 to 150 days) and is ideally suited to tropical and subtropical regions. It grows best on fertile, well-drained soils, preferably light sandy loams. Although sesame is highly resistant to drought, it is not considered a poor-land crop. Areas where the rainfall is sufficient for the production of cotton are considered adequate in moisture for sesame without resorting to irrigation. High yields have been reported in the United States from experimental plots grown under irrigation in desert areas.

Sesame is planted and cultivated as a row crop, requiring about 1 pound of seed (160,000 seeds) per acre. The plant will emerge in three to five days and the rate of growth for the first three weeks is quite slow. During this critical period, weeds must be controlled carefully. This requires considerable hand labor; however, tests at the Texas Research Foundation have pointed out the feasibility of the application of certain pre-emergent herbicides to the sesame seedbed. At four weeks of age the stand is usually well established, and the plants exhibit rapid growth with a good tolerance to drought and high temperatures.

b. Diseases and Insect Pests

Several diseases have been reported as attacking the sesame plant during various stages of growth. Fortunately they have not become a serious problem in most of the Western Hemisphere. As sesame is cultivated on a more extensive scale, the probability of serious disease damage cannot be minimized. The fungi *Cercospora sesami* Zimm. (15), an *Alternaria* species, and one bacterium, *Pseudomonas sesami* Malkoff, are known to cause leaf spot diseases whose development and spread are promoted by heavy rainfall and high temperatures. Damage from leaf spot disease normally will not become a serious threat throughout the semiarid regions if properly treated seed is planted.

Thomas (16) has reported that bacterial leaf spot can be controlled by soaking the infected seeds in an aqueous solution of streptomycin. Armstrong and Armstrong (17) tested the effects of fusarium wilt, *Fusarium oxysporum* f. *sesami* Castellani, on twelve varieties of sesame for wilt resistance, Sirogoma proving highly resistant. Other diseases which have been reported to attack sesame are: a bacterial wilt, *Pseudomonas solanacearum* E. F. S.; southern blight, *Pellicularia rolfi* Sacc.; charcoal rot, *Macrophomina phaseoli* Taub.; and cotton root rot, *Phymatotrichum omnivorum* (Shear) Duggar.

Aphids have caused extensive damage to sesame; leaf miners, chinch bugs, and boll worms may cause variable amounts of destruction. When the sesame plant is the main source of green vegetation, grasshoppers can be a definite hazard.

2. Harvesting

In the majority of countries producing the bulk of sesame, harvesting is a manual-labor operation. The sesame plants are cut by hand after the leaves have dropped, and the plants are bundled and shocked until dry. During the drying process the seed capsules open, and the seeds are removed by shaking the inverted plant over a suitable receptacle or blanket.

In areas where the cost of farm labor is an important item, competition with other oilseed crops makes mechanical harvesting mandatory. Such an approach has been studied in the United States since the early 1950's. The grain or row binder method of harvesting dehiscent sesame appears to yield consistent and satisfactory results. This method entails the cutting and bundling of sesame plants after the leaves have dropped, shocking and tying the bundles, and combining from the shock after the plants have dried. A wooden or metal platform attachment in front of the cutter bar of the combine reduces the amount of hand labor to the minimum. After proper combine adjustments have been made, three men can usually thresh one acre of sesame per hour with a seed recovery of up to 95%.

Concentrated efforts are being directed to the establishment of profitable and efficient methods of harvesting the new indehiscent strains of sesame. Delay in reaching maturity and the loss of seed in combining due to incomplete threshing of the closed pods are the paramount obstacles. Although present-day combines are not adequate in all respects for threshing indehiscent sesame, they will satisfactorily harvest the crop. Employing an Allis Chalmers Model 66 combine in a threshing test conducted at the Edisto Experiment Station at Blackville, South Carolina, Park* found that seed yield decreases seriously at cylinder speeds of less than 1000 r.p.m., and germination decreases significantly at speeds higher than 800 r.p.m. Collister (11) has presented an excel-

* J. K. Park, personal communication, 1956.

lent summary of harvesting procedures. The development of strains of indehiscent sesame with "paper-shell"-type pods would be a distinct aid toward more efficient harvesting techniques.

V. PROCESSING

Processing of sesame seed on a commercial basis is chiefly by means of continuous screw presses which have superseded the hydraulic method of oil extraction and may be replaced eventually by the prepress solvent-extraction process. In many agricultural areas of the world, however, sesame oil is extracted by primitive methods.

Data are not available on the percentages of sesame seed processed by the various extraction methods; however, the world extraction rate for commercial sesame crushings is estimated at 45%. In India 25% of the seed reserved for crushing is processed commercially; the remainder, 75%, is crushed in village *ghanis* (Fig. 3, page 603). (For a general description of oilseed processing, see Chapter 4.)

1. Screw-Pressing

The time and temperature relationship in the cooking process prior to the extraction of sesame oil is not standardized. Physical condition, moisture content, and variety of seed are all contributing factors to these variations in crushing procedures. The practice of cleaning and adjusting the moisture content of the seed prior to crushing is common among screw-press operators, for considerable damage to the barrels of the press has occurred when seed contaminated with sand or other abrasive material was processed. From this point on, differences in operating techniques are encountered. In general, controllable temperatures do not exceed 240°F. at any stage of the cooking process, but the temperature of the material within the barrel is subject to wide fluctuations. The quality of the oil and meal are dependent on these time and temperature variables during cooking and pressing; and critical studies of these factors are a neglected phase that requires technological attention.

An average screw press such as the V. D. Anderson Super Duo Expeller will process 13 to 20 tons of sesame seed per day with recoveries of oil and meal (containing 5% oil) of 47% and 52%, respectively.

2. Prepress Solvent Extraction

The trend in sesame processing is toward prepress solvent extraction. This method is currently employed in parts of the United Kingdom and undoubtedly will be adopted elsewhere, wherever feasible. The seed is dried to about 4% moisture prior to prepressing, care being

exercised to avoid over-rolling before prepressing. Pilot-plant tests conducted at the V. D. Anderson Company in the United States indicated that prepress cakes containing 8 to 12% oil could be produced at a rate equivalent to 40 to 50 tons of sesame seed per day by the Super Duo Expeller (18). The cake, after prepressing, is granulated, conditioned, and flaked, and then is extracted with solvent by conventional solvent-extraction methods to produce meals of 0.5% oil content. Experimental data have disclosed that the flaked cake from 180 tons of sesame seed can be processed in 24 hours in a solvent-extraction unit of the size capable of handling 100 tons of soybeans per day.

3. Filtration-Extraction

Preliminary bench-scale tests (18) on the evaluation of the filtration-extraction process for sesame seed have been conducted at the Southern Utilization Research Division, U. S. Department of Agriculture. Preparation and extraction variables were studied, and it was concluded that extraction efficiencies of approximately 99% could be obtained.

VI. SEED AND OIL

1. Seed

An average analysis of sesame seed would indicate 25% crude protein, 50% ether extract, 4% crude fiber, 5% ash, 11% nitrogen-free extract, and 5% moisture.

Small quantities of hulled seed are used as toppings for breads and confectionary items. The ancient role of sesame seed in the diets of many millions of Asian peoples is well known. A product called "carate de ajonoli," a suspension of toasted sesame seed in a sweetened solution of water or milk, is popular in Venezuela. Uses as human food are discussed in Chapter 9.

2. Oil

Sesame oil is an excellent vegetable fat and is widely used as a salad or cooking oil and in shortenings and margarines. In both the unhydrogenated and hydrogenated forms, sesame oil is considered nutritionally equivalent to other edible vegetable fats. It is devoid of vitamin A and is not a prominent source of vitamin E. Sesame oil is recognized for its marked stability to rancidity and its desirable flavor. Andraos *et al.* (19) presented data on the extraction, the processing, and the physical and chemical characteristics of oil obtained from white sesame seeds. Menezes *et al.* (20) investigated the properties of the solvent-extracted oils from four varieties of sesame seed and found only slight variations in the composition of the oils. The stability of sesame oil is attributed

to the presence of sesamol, the methylene ether of oxyhydroquinone. Crude sesame oil contains about 0.1 to 0.2% of sesamol in the bound form, with only traces of free sesamol. Hydrogenation and bleaching increase the free sesamol content of the oil and account for the increased stability of the hydrogenated and bleached products. Deodorization of the oil removes the free sesamol, resulting in a significant reduction in stability (21).

Sesame oil is also used in the manufacture of soap, as a fixative in the perfume industry, and as a vehicle for fat-soluble substances in pharmaceutical products. Sesame oil exerts a synergistic effect when added to insecticide sprays containing pyrethrins and rotenone. Budowski and Markley (22) have written an outstanding review on the chemical and physiological properties of sesame oil.

VII. SESAME MEAL AND PROTEIN

The nutritive requirements of non-ruminants are more critical than those of ruminants. In the formulation of nutritionally adequate rations for non-ruminants, additional quantitative data on the essential nutrients present in nitrogenous feed ingredients, other than the usual percentages of protein, fat, fiber, ash, and moisture, are required. The poultry nutritionist, for example, needs to know the levels of essential amino acids, vitamins, minerals, and energy present in the majority of feedstuffs used in poultry rations.

1. General Composition of Meal

Table III lists the average analysis of three lots of screw-pressed sesame meal produced in Mexico and used in the manufacture of poultry concentrates by the Andersen-Smith Milling Co., San Francisco, California.

2. Amino Acids

Critical shortages of animal protein concentrates during World War II prompted the search for suitable vegetable protein concentrates which could serve as supplements to soybean oil meal, particularly in poultry rations. Soybean meal, when used as the sole protein source in the diet of the growing chick, is adequate in all amino acids for this species except methionine. Investigations on the mutual supplementary effect of sesame meal and soybean meal were initiated at the Poultry Husbandry Division, University of California, during the early 1940's. Almquist and Grau (23) found that best gains were made by chicks fed a ration in which the ratio of sesame-soybean protein approached 7:13, all the protein being furnished by sesame and soybean meals at a total

protein level of 20%. When sesame meal was fed as the only source of protein, poor chick growth was attained, indicating an amino acid deficiency. Further biological tests disclosed lysine to be the primary deficiency of sesame protein. A subsequent paper by Grau and Almquist (24) proved that optimal chick growth was attained when 0.5% L-lysine was added to an all-sesame protein diet. These workers concluded that sesame meal is capable of supporting optimal chick growth when fed in conjunction with protein concentrates rich in lysine. The

TABLE III
AVERAGE ANALYSIS OF THREE LOTS OF SCREW-PRESSED SESAME MEAL^a

Component	Average	Range
	(%)	(%)
Crude protein (N × 6.25)	45.87	43.87-47.11
Ether extract	7.55	8.85- 6.07
Crude fiber	5.38	5.57- 5.09
Ash	11.82	12.24-11.54
Nitrogen-free extract	23.86	24.74-21.92
Moisture	5.52	6.52- 4.59
Calcium	2.29	2.46- 2.12
Phosphorus	1.39	1.42- 1.38
	mg./lb.	mg./lb.
Manganese	19.4	22.7-17.3
Riboflavin	2.1	2.2- 2.0
Niacin	50.4	52.2-47.6
Pantothenic acid	4.1	4.8- 3.0

^a From the Analytical Laboratory, Andersen-Smith Milling Co., San Francisco, Calif.

conclusions reached by the California investigators were verified when Block and Bolling (25) published their amino acid analysis of sesame seed protein.

Table IV illustrates the adequate essential amino acid composition, with the exception of lysine, of sesame seed protein in comparison with the protein of beef and casein. Table V summarizes analytical data on the amino acid composition of crude sesame protein. A comparison of the amino acid composition of sesame meal and two competitive oilseed products, soybean and cottonseed meal, is presented in Table VI. Sesame meal contains over two times as much methionine as do the others; in fact, sesame meal surpasses all other oilseed meals as a source of methionine. If ample supplies of sesame meal, along with lysine-rich oilseed meals, are available, feed manufacturers can produce broiler and other poultry rations without recourse to animal protein concentrates or synthetic DL-methionine. Since animal protein concentrates

TABLE IV
AMINO ACID ANALYSES OF SESAME SEED, CHOPPED BEEF, AND CASEIN PROTEINS^a
(Calculated to 16.0% nitrogen)

Amino acid	Sesame seed (%)	Chopped beef (%)	Casein (%)
Arginine	8.7	7.7	4.1
Histidine	1.5	2.9	2.5
Lysine	2.8	7.2	7.5
Tyrosine	3.5	3.4	6.4
Tryptophan	1.8	1.3	1.2
Phenylalanine	8.3	4.9	5.2
Cystine	1.3	1.3	0.4
Methionine	3.1	3.3	3.5
Threonine	3.6	5.4	3.9
Leucine	7.5	7.7	12.1
Isoleucine	4.8	3.0	6.5
Valine	5.1	3.5	7.0
Glycine	9.3		

^a According to R. J. Block and D. Bolling, *Arch. Biochem.* **6**, 277 (1945).

TABLE V
AMINO ACID CONTENT OF SESAME PROTEIN
(Grams of amino acid per 16 g. of nitrogen)

Amino acid	a (g.)	b (g.)	c (g.)
Arginine	8.7	10.04	11.91
Histidine	1.5	2.36	2.21
Lysine	2.8	2.66	2.76
Tryptophan	1.8	1.22	1.91
Phenylalanine	8.0	4.48	4.73
Methionine	3.2	2.03	2.65
Threonine	4.0	3.49	3.64
Leucine	7.5	6.12	6.92
Isoleucine	4.8	3.56	4.27
Valine	5.1	4.82	5.06
Tyrosine	3.5	4.70	
Cystine	1.3		
Glycine	9.3		
Alanine	3.5		

^a According to R. J. Block and D. Bolling, "The Amino Acid Composition of Proteins and Foods," 2nd ed., p. 491, Charles C Thomas, Springfield, Ill., 1951.

^b According to H. H. Williams, *Cornell Univ. Agr. Expt. Sta. Mem.* **337**, 31 pp. (1955).

^c According to C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).

are normally in short supply and usually high in cost per unit of protein, the availability of sesame meal to the feed-manufacturing industry can be of considerable economic importance, particularly since many of the unidentified growth factors contributed by animal protein sources are available in concentrated forms and can be added to vegetable protein rations.

Synthetic DL-methionine is available in ample quantities and at a reasonable price and has been widely used in the United States as an ingredient of broiler rations and dog foods. Processors of sesame seed

TABLE VI
AMINO ACID CONTENT OF SESAME, SOYBEAN, AND COTTONSEED MEAL^a

Amino acid	Sesame meal, 46.08% crude protein (%)	Soybean meal, 45.29% crude protein (%)	Cottonseed meal, 39.61% crude protein (%)
Arginine	5.49	3.38	4.38
Histidine	1.02	1.12	1.07
Lysine	1.27	2.79	1.67
Tryptophan	0.88	0.76	0.63
Phenylalanine	2.18	2.20	2.09
Methionine	1.22	0.63	0.59
Threonine	1.68	1.82	1.38
Leucine	3.19	3.48	2.46
Isoleucine	1.97	2.49	1.59
Valine	2.33	2.45	1.98

^a According to C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).

should recognize the economic importance of synthetic methionine in non-ruminant nutrition before any attempt is made to justify an increase in the price of sesame meal over competitive oilseed meals, based solely on the higher methionine level of sesame meal. (See also discussion in Chapter 13.)

3. Vitamins and Minerals

Sesame meal contains little, if any, vitamin A activity, and is not considered a good source of vitamin E. The riboflavin content of sesame meal (2.1 mg./lb.) is similar to the published values for soybean and cottonseed meal; the average pantothenic acid value (4.1 mg./lb.) tends to be lower. Jukes (26), employing a biological assay with chicks, found sesame meal to contain 6 γ of pantothenic acid per gram (2.7 mg./lb.). Sesame meal contains more niacin (50.4 mg./lb.) than the commonly

used oilseed meals, with the exception of peanut meal. It is difficult to arrive at an average value for thiamine content, as there is considerable variation in the amount destroyed during processing, depending on the time and temperature relationship during cooking and pressing. Cravioto *et al.* (27) reported a thiamine value of 1.36 mg. per 100 g (6.2 mg./lb.) for sesame seed, a thiamine content similar to that of soybeans. Two samples of screw-pressed sesame meal were found to contain 673 and 700 mg. of choline per pound (28).

Sesame meal is an excellent source of minerals, particularly calcium and phosphorus. The relatively high calcium and phosphorus content of sesame meal, 2.29 and 1.39% respectively (Table III), and the favorable calcium-phosphorus ratio place sesame meal in a unique position among commercial oilseed meals. Levels of three other essential minerals in sesame meal are: magnesium, 0.78%*; potassium, 1.35% (29); and manganese, 19.4 mg./lb. (Table III). Data on calcium, niacin, and riboflavin contents of sesame meals prepared from ten varieties of seed have been reported by Lease (30).

4. Digestible Protein, Total Digestible Nutrients, Productive Energy

Folger (31), from the results of a digestion trial with sheep, found the following digestible nutrients per 100 pounds of sesame meal: 35.8 pounds of digestible crude protein, 8.1 pounds of digestible ether extract, 23.2 pounds of digestible carbohydrates, and 77.2 pounds of total digestible nutrients (TDN). Schneider (32) reported an average value of 36.7% digestible crude protein and 70.8% total digestible nutrients for sesame meal (average of sixteen different digestion trials). For the growing chick, Fraps (33) determined, by means of slaughter experiments, the productive energy of a sesame meal containing 41.6% crude protein and 12.5% ether extract. A productive energy value of 706 Calories per pound of meal was found; this value is strictly applicable to the sesame meal tested and not necessarily valid for sesame meals of different chemical analyses.

5. Growth Inhibitors

Sesame seed is not known to contain proteolytic enzyme inhibitors, and no reports could be found indicating the presence of any substance in sesame meal having an adverse effect on the growth of poultry or swine, egg production, and egg quality. Patrick (34) investigated the effects of the addition of various supplements to a ration composed of 65% ground grains, 30% sesame meal, vitamins, and minerals on the growth and feather pigmentation of day-old New Hampshire chicks.

* R. W. Caldwell, Andersen-Smith Milling Co., unpublished data, 1947.

The addition of 0.5% DL-lysine improved growth and feather pigmentation; however, further improvement in growth and feather pigmentation occurred when 0.5% DL-lysine with either 10% soybean oil meal or 5% albumin was added to the basal diet. Patrick concluded that, in addition to lysine, sesame meal is deficient in a factor(s) found in soybean oil meal and albumin. Squibb and Braham (35) showed that good growth and feed conversion were obtained with New Hampshire chicks when 0.45% L-lysine was added to an all-vegetable protein ration containing 55.5% ground corn and 40.0% sesame meal.

6. Effects of Overheating

In addition to the deleterious effects of prolonged heat treatment on the stability of heat-labile vitamins, excessive heat treatment can impair the availability of certain amino acids. Two types of amino acid impairment caused by the overheating of food proteins are possible (36). Drastic heat treatment of protein concentrates containing little free carbohydrate causes the amino acid lysine and possibly other amino acids to be tied up by new chemical linkages to other amino acids with a net result of reduced availability to the animal. In addition to this type of heat impairment, the overheating of protein concentrates containing significant quantities of free carbohydrates results in a reaction of the amino acids lysine, arginine, and tryptophan with carbohydrates, which produces combinations that prevent the complete regeneration of these amino acids in the digestive tract. In order to ensure the full nutritional value of lysine, arginine, tryptophan, and the heat-sensitive amino acid cystine, suitable precautions must be observed to avoid the overheating of sesame meal during processing. (See also a general discussion of the effect of heat on protein in Chapter 5.)

7. Sesame Protein

Studies dealing with the physical and chemical properties of sesame protein are not numerous. Arthur and Volkert (37) found sesame protein comparable to cottonseed protein with respect to solubility in aqueous media, viscosity of alkaline dispersions, and color. Their data indicate that sesame protein probably can be used in the manufacture of certain glues and sizes, where color is not a deterrent.

VIII. USES OF SESAME MEAL

1. Feeding Ruminants

The major part of the sesame meal produced in the world is used in the nutrition of ruminants. This is particularly true in areas where

sesame meal is the only oilseed meal available or the higher cost of competitive oilseed meals favors the use of sesame meal for this purpose. It is doubtful whether sesame meal will be widely used in the feeding of dairy and beef animals or sheep as long as equivalent quantities of digestible protein and total digestible nutrients are available from other sources at a lower cost.

2. Feeding Poultry

The importance of sesame meal as an ingredient of poultry rations has been stressed in previous sections of this chapter. Its potential for use in feeds for poultry, particularly in the Western Hemisphere, is encouraging. The American Feed Manufacturers Association, Chicago, Illinois, released statistical data on the volume of manufactured feeds produced in the United States for 1955. Out of a total of 33,600,000 tons of manufactured feeds, poultry feeds accounted for 19,992,000 tons of which 5,757,696 tons were for broiler feeds, the type of feed that could use sesame meal to best advantage. On the assumption that these broiler feeds contained 5.0% of sesame meal, 287,885 tons of sesame meal would have been required. This amount of meal is equivalent to approximately 560,000 tons of sesame seed, roughly 26% of the world production in 1953. Higher levels of sesame meal could be used in broiler feeds, and there are no nutritional restrictions to prevent the use of sesame meal in other types of poultry feeds.

Almquist (38) reported on the results of some broiler experiments employing all-vegetable protein diets (22% crude protein). The variables studied were soybean oil meal, sesame meal, and DL-methionine with a broiler ration containing 6% fish meal, 8.0% sesame meal, and 13.3% soybean oil meal serving as the control. The other ingredients of the diets were identical, and all rations contained antibiotic feed supplement. The results of this large-scale test are summarized in Table VII. It is evident that the methionine contributed by the sesame meal was fully equivalent to synthetic methionine, and that satisfactory results were obtained with an all-vegetable broiler ration containing soybean oil meal and sesame meal; however, significantly better gains and feed conversions were realized on the fish meal control diet. The economic relationship between diets based on a combination of soybean oil meal and sesame meal and those based on soybean oil meal and synthetic methionine cannot be ignored. From the data given in Table VII, as an example, the use of sesame meal in all vegetable protein rations could not be justified if the cost of the soybean oil meal fortified with synthetic methionine were less.

TABLE VII
RESULTS FROM BROILER CHICK TESTS WITH ALL-VEGETABLE PROTEIN DIETS
AND WITH FISH MEAL DIETS^a
March 1950, New Hampshire, mixed sex

Diet	Variables in diet (%)	Number of chicks	Average weight at 10 weeks (lb.)	Feed conversion (feed/gain)	Methionine in diet (%)
1	Soybean meal 32.6	2000	2.921	3.09	0.37
2	Soybean meal 32.6 Methionine 0.13	4000	3.044	2.82	0.50
3	Soybean meal 32.6 Methionine 0.18	4000	3.044	2.79	0.55
4	Soybean meal 21.0 Sesame meal 11.7	2000	3.051	2.83	0.45
5	Soybean meal 21.0 Sesame meal 11.7 Methionine 0.05	2000	3.055	2.81	0.50
6 ^b	Fish meal 6.0 Sesame meal 8.0	2000	3.224	2.67	0.51
7 ^b	Fish meal 6.0 Sesame meal 8.0 Methionine 0.05	2000	3.200	2.68	0.56

^a According to H. J. Almquist, *Proc. Semi-Annual Meeting Nutrition Council Am. Feed Mfrs. Assoc.* Chicago, Ill., Nov. 30-Dec. 1, p. 9 (1953).

^b Fish meal diets contained 13.3% soybean meal.

3. Human Nutrition

Although sesame seed has served as a valuable nutritional adjunct to the diets of sections of the world's population, several health organizations are interested in programs aimed at the more efficient use of the protein from sesame seed (39).

Jaffé (40, 41) conducted a series of rat feeding trials for the biological evaluation of sesame meal alone, and in various combinations with corn meal and peanut meal. On the basis of encouraging results of the rat feeding tests, *arepas* (local corn breads) were prepared with various amounts of toasted sesame seed. A taste panel preferred the arepas containing 10% sesame seed. Limited quantities of this type of arepas were offered for sale in the local markets of Maracay, Venezuela,

and the new product was well received by the public. Arepas were also prepared with corn meal and a mixture of a finely ground meal containing 80% sesame meal and 20% peanut meal.* Arepas with 5, 10, and 20% of the sesame meal-peanut meal mixture were prepared, and the flavor of all samples submitted to a taste panel was judged excellent. Arepas enriched with sesame meal was never produced in large quantities in Venezuela, however.

TABLE VIII

AMOUNTS OF THE ESSENTIAL AMINO ACIDS FURNISHED BY 1 OUNCE OF SESAME MEAL AND THEIR RELATIONSHIP TO THE TENTATIVE MINIMUM DAILY REQUIREMENTS OF MAN

Amino acid	Amount of amino acid from 1 ounce of sesame meal (g.)	Tentative minimum daily requirement ^a (g.)	Per cent of minimum daily requirement (%)
L-Tryptophan	0.23	0.25	92.0
L-Phenylalanine ^b	0.60	1.10 ^b	54.5
L-Lysine	0.36	0.80	45.0
L-Threonine	0.46	0.50	92.0
L-Methionine ^c	0.37	1.10 ^c	33.6
L-Leucine	0.89	1.10	80.9
L-Isoleucine	0.51	0.70	72.8
L-Valine	0.65	0.80	81.3

^a According to W. C. Rose, R. L. Wixom, H. B. Lockhart, and G. F. Lambert, *J. Biol. Chem.* **217**, 987 (1955). Values determined with diets containing the eight essential amino acids and sufficient extra nitrogen to permit synthesis of the non-essentials. See also *Nutrition Revs.* **14**, 232 (1956) and discussion in Chapter 2.

^b This value was obtained with diets which were devoid of tyrosine.

^c This value was determined with cystine-free diets. Cystine can exert a sparing effect of 80 to 89% on the minimal methionine requirement.

Since man can derive his essential amino acid requirements from vegetable protein sources as well as from animal proteins, it is of interest to consider the quantities of essential amino acids contributed by 1 ounce (28.35 g.) of a sesame meal containing 45% crude protein. The amounts of the essential amino acids furnished by 1 ounce of sesame meal and their relationship to the tentative minimum daily requirements of these amino acids for normal man, when sufficient extra nitrogen for the synthesis of non-essentials is provided in the diet, are presented in Table VIII. One ounce of sesame meal will supply the following percentages of the recommended daily dietary allowances for a

* W. Jaffé, personal communication, 1955.

physically active man: protein, 18.2%; calcium, 65.0%; and niacin, 20.9%.

Besides the psychological problems associated with the introduction of a new food product to the established diets of different populations, the effects of the various methods of processing on the palatability, nutritive, and keeping qualities of sesame meal will have to be investigated. Since many nutritionists regard oxalates as a suspect material in the field of human nutrition, decortication of sesame seed may be necessary as the *outer epiderm* of the seed contains calcium oxalate (42). The hull contains approximately 3% oxalate.

IX. TRENDS

It would be hazardous to predict categorically trends for an oilseed which is barely approaching the economic and technological impact of large-scale, modern, commercial possibilities. It would be more judicious to predict the following probable trends on a tentative basis only:

1. Research on the development of improved indehiscent strains of sesame of high seed yields will be pursued vigorously. Considerable attention will be focused on improving harvesting machinery and methods.

2. Sesame oil will be accepted readily by the manufacturers of salad oils, shortenings, and margarines, although vigorous competition will be offered by other well-established, edible vegetable oils.

3. Owing to higher oil yields and more efficient production rates of oil and meal, the adoption of the prepress solvent-extraction method of processing sesame seed is imminent. The employment of this processing method guarantees the production of sesame meals containing 50% protein, thus improving the competitive position of the meal.

4. In areas where large tonnages of poultry feeds are manufactured, considerable sesame meal may be used, depending, of course, on the competitive relationship of sesame meal with other oilseed meals, and with due regard to the importance of synthetic methionine. Limited quantities of sesame meal may also be used in swine rations.

5. Some sesame meal may be used for the enrichment of the diets of populations where sesame is the main oilseed crop, and economic factors prohibit extensive use of animal proteins.

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CHAPTER 19

SUNFLOWER SEED OIL MEAL

D. R. CLANDININ

I. INTRODUCTION

The sunflower (*Helianthus annuus*) originated in Latin America; however, it was first developed as an oilseed crop in southeastern Europe. Sunflowers are cultivated mainly for oil; the better sunflower oils are used in the manufacture of margarine, shortening, and salad oils, and the poorer quality oils are used in soaps, paints, varnishes, and lubricating oils. The seed contains 20 to 32% oil, and, when decorticated before extracting, the residue makes a high-quality protein feed-stuff suitable for incorporating in poultry and livestock feeds.

II. PRODUCTION AND TRADE

1. Acreage

According to figures released by the Commonwealth Economic Committee (1), 15.3 million acres of land were devoted to the production of sunflower seed in the crop year 1953-54 in the principal sunflower-producing countries, compared with the record crop acreage of 17.4 million acres in 1950-51 and the 1938-39 figure of 9.9 million acres. In 1953-54 the Soviet Union had the largest acreage (9,400,000 acres) devoted to the production of sunflower seed; Argentina was the second most important producing country, having 1,413,000 acres in sunflower production. The Soviet Union figure represents a 600,000-acre increase over the 1950-51 acreage, whereas the Argentine figure constitutes a 2,600,000-acre decrease from the 1950-51 acreage. Other important producing countries in 1953-54 were Uruguay (398,000 acres), South Africa (370,000 acres), Turkey (295,000 acres), Yugoslavia (240,000 acres), and Chile (120,000 acres). Ethiopia also ranks as a major sunflower-producing country; the estimated area sown in 1950-51 was 450,000 acres. Several Balkan countries also produce sunflower seed on a large scale, but little information on recent crop acreage is available. The Commonwealth Economic Committee esti-

mated Roumania at 1,340,000 acres in 1948, Hungary at 711,000 acres in 1949, and Bulgaria at 378,000 acres in 1947. The Committee suggests 3,000,000 acres as a reasonable figure for acreage sown to sunflowers in Ethiopia and these Balkan countries in 1953-54.

2. Yield

The major production of sunflower seed in 1953-54 occurred, as might be expected, in the Soviet Union, the Balkan countries, and Argentina. World production (1) of sunflower seed in 1953-54 was approximately 4.7 million tons compared with the record crop of 5.0 million tons in 1950-51 and the 1938-39 figure of 3.0 million tons. World production of sunflower seed, excluding the Soviet Union and eastern Europe, amounted to only 0.9 million tons in 1953-54, as against 1.7 million tons in 1950-51; however, the 1953-54 figure of 0.9 million tons was more than twice the prewar (1938-39) level.

The yields of sunflower seed and of sunflower oil per acre vary, depending on the variety grown, the climate and soil, and cultivation practices employed. Yields of up to 1600 pounds to the acre are not uncommon, although yields of 350 to 600 pounds per acre are considered average (2). Based on world acreage sown and world production of sunflower seed in the crop years 1949 to 1954 inclusive (1), the yield of sunflower seed per acre amounted to 572 pounds.

3. Trade

Exports of sunflower seed during 1953 (73,000 tons) were greater than in any previous postwar year, nearly all of which was shipped from eastern Europe. World exports of sunflower oil in 1953-54 amounted to 34,500 tons, which was well below the 1950-51 high of 126,500 tons when Argentina production and shipments were at a high level. The chief countries exporting sunflower oil in 1953-54 were Argentina, Uruguay, and Hungary (1).

In 1948-52 the United Kingdom was the chief importer of both sunflower seed and oil but purchased only a small quantity in 1953. Western Germany was the major importer of seed in 1953, and western Europe and Chile accounted for the great bulk of oil imported in that year (1).

4. Comparative Value of Products

On the basis of an average yield of 572 pounds of sunflower seed per acre (1), an average oil extraction of 27%, a decorticated sunflower seed oil meal yield of 35%, and a hull yield of 38%, which figures take into account the 20 to 32% oil and 35 to 45% hull content

of sunflower seed, the average yield per acre of oil, meal, and hulls would amount to 155, 200, and 217 pounds, respectively. From 1955 Canadian prices of sunflower oil (16 cents per pound), decorticated sunflower seed oil meal (4 cents per pound), and hulls as litter (0.5 cent per pound), the value of oil, meal, and hulls per acre would be \$14.80, \$8.00, and \$1.08, respectively.

III. SUNFLOWER SEED

1. Appearance and Structure

The sunflower seed (achene) is more or less four-sided and flattened. It has a dry, brittle, black or striped grey and black pericarp

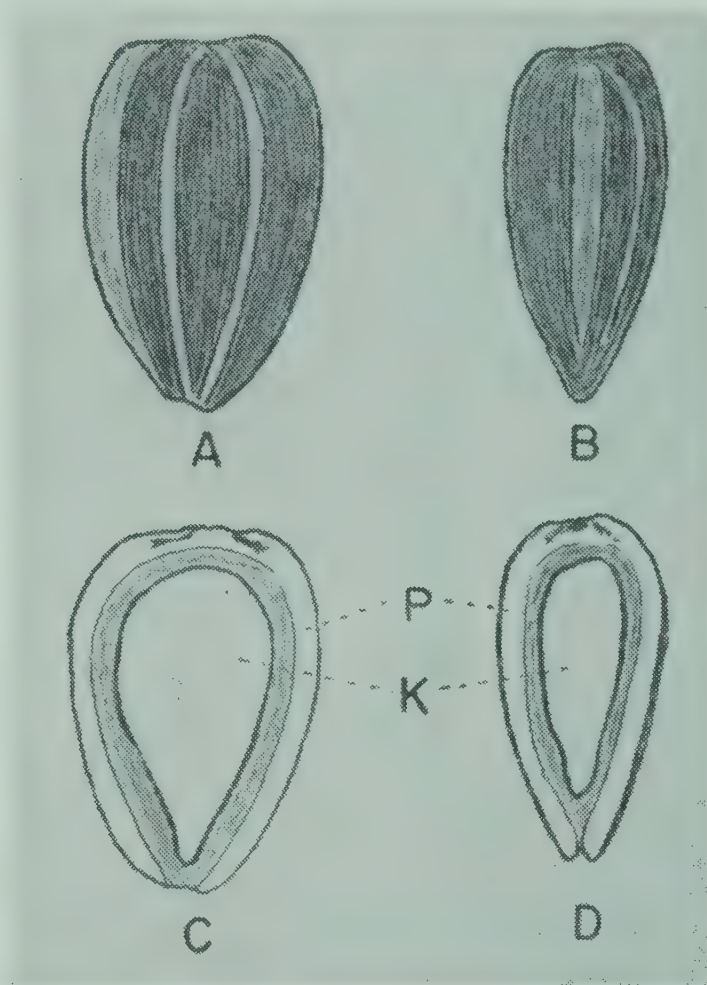


FIG. 1. Sunflower seed (achene). *A* and *B*, external views; *C* and *D*, sectional views; *P*, pericarp or hull; *K*, kernel.

which consists of five fairly distinct layers and encloses a whitish kernel (Fig. 1).

In certain strains of sunflower, a so-called "armored coat" consisting of a phytomelanin deposit is found which prevents the larva of the sunflower moth, *Homeosoma nebulella*, from penetrating the pericarp to the kernel. The armored coat is found in both light- and dark-colored seeds and is a selection factor in some sunflower improvement programs (3).

Oil and protein in sunflower seed are located mainly in the kernel; the pericarp consists largely of fiber. It has been shown that the peri-

carp or husk develops to full size and then, after fertilization, is gradually filled with kernel. There is a considerable increase in dry matter, oil, and protein content and a slight decrease in husk content as ripening proceeds. The best kernel development and highest content of oil and protein are achieved when the seed is fully ripe.

2. Varieties

Many different varieties and strains of sunflower are grown. In the U.S.S.R. three main types of seed are recognized (3).

a. The chewing kind, which has a large seed, a thick hull, and a loose kernel.

b. The oil sunflower type, which has a small seed and a thin hull which tightly fits the kernel.

c. The dual-purpose kind, which is considered to be intermediate between these two groups in hull thickness.

At the present time extensive breeding programs are under way in the U.S.S.R. (3-5) and in other parts of the world (6, 7) to develop varieties, strains, and crosses which possess the following characteristics: earliness; high yield; high quality, including (1) low percentage hull, (2) high oil content, and (3) high protein content; desirable morphology, including (1) moderate height, (2) single head, and (3) flat-type head; and resistance to disease. In general, in these breeding programs the use of varietal selection, intervarietal crosses, the hybrid corn-breeding technique, and the search for crosses yielding significant combining ability has produced favorable results.

Promising varieties and strains that have been developed (3) in the U.S.S.R. for growing in southeastern Russia are: Saratov 169, Puksinka 10 and 3, Kruglik A-41, and Cernjanka 31, which show a fair degree of resistance to broom rape (*Orobanch*); Zdanov 8281 and 6432 and Puksinka 62 and Zelenka 61, which show good resistance to this disease; and Armavir 1846 and 1813 and VNIIMK 3519, 4036, 6540, 4966, and 4418, which are noted for their high oil content. Saratov II-10 has also been reported (5) to be a high yielder and to have a high oil content. Numerous varieties superior to Siberian Pioneer have also been developed (4) in the U.S.S.R. for growing in western Siberia, notable among which are Barnaul 2296, 3196, 2924, and 2911.

In Argentina, the principal varieties grown are Klein, Buck, and Saratov; in South Africa, another significant producer of sunflower seed, the varieties cultivated are Juniper, Giant Russian, and Ella. Varieties grown in some of the countries where minor production of sunflower seed occurs are: French Morocco—Juniper and Sunrise; Kenya—Giant Black; Canada—Sunrise, Advance, and Beacon.

Australia—Giant Russian, Mennonite, and Sunrise; and the United States—Large Seeded Russian, Greystripe, Black Manchurian, and Arrowhead.

3. Composition

The chewing kind of sunflower seed has a large seed, a thick hull, and a loose kernel. In gross composition it runs 40 to 50% hull, 20 to 25% oil, and yields 25 to 35% decorticated sunflower seed oil meal. The oil type of sunflower seed has a small seed and a thin hull which tightly fits the kernel. In gross composition it runs 35 to 45% hull, 25 to 32% oil, and yields 30 to 40% decorticated sunflower seed oil meal. These variations in seed composition are accounted for on the basis of varietal and strain differences and differences in soil and climatic conditions under which the crops are grown (4-6).

In southeastern Russia, in tests (5) involving a series of sunflower varieties and strains grown under similar conditions, variations in oil content of the seed from 28.9 to 38.8% and in hull content from 28.8 to 40.5% were found. In addition it was shown that the oil and hull content of sunflower seed could be markedly affected by selection. In this regard, as a result of seven years of selection, the oil content of seed of Saratov II-10 was increased from 36.6 to 42.3%, and the hull content was decreased from 37.7 to 29.0%. In tests in western Siberia (4) variations in gross composition of seed of different varieties and strains of sunflower grown under similar conditions have also been demonstrated, and several strains of seed high in oil and low in hull content have been produced by the breeding techniques referred to previously.

Tests conducted in central Europe (6), in addition to showing effects of variety, strain, and selection on gross composition of sunflower seed, have demonstrated clearly that the soil and climatic conditions under which the sunflower is grown also influence gross composition. On the heavier soils and in the northern areas the percentage hull in the seed was higher than in seed grown on the lighter soils and in the southern regions. Suboptimal precipitation and drought were found to decrease the oil content of the seed.

IV. PROCESSING

1. Method

The meat of the sunflower seed, as previously indicated, is surrounded by a brittle hull or pericarp. This hull constitutes 35 to 45% of the weight of the sunflower seed and must be removed before process-

ing if maximum yield of oil and a meal of high feeding value are to be obtained. If the hull is not removed before processing, a meal of excessively high fiber content and consequently of questionable feeding value for monogastric animals and birds is produced.

In one Canadian plant (Co-op Vegetable Oils Limited, Altona, Manitoba) the processing method employed (8) consists of removing the hulls from the seeds by a special dehuller and separating the hulls from the meats by means of shaking screens. The relatively hull-free meats are next cooked for $\frac{1}{2}$ hour at 240°F., conditioned for about 3 minutes at 260°F., and then passed through Anderson expellers (screw presses). By this extraction procedure meals containing as low as 4% oil are obtained. Where a more complete extraction of oil is sought, screw-pressing to remove the bulk of the oil followed by solvent extraction to reduce the residual oil below 1% is a suitable extraction technique. (For a general discussion of oilseed processing see Chapter 4.)

2. Effects on Nutritive Value of Meal

The importance of removing all or most of the hulls from the seeds before screw-pressing with regard to the feeding value of the resultant meal for non-ruminants has already been mentioned. From the experience of the writer it would appear that meals should not contain more than 10 to 12% fiber. Meals containing lower levels of fiber than this, of course, should prove more satisfactory from the nutritional point of view.

The amount of heat used in processing sunflower seed oil meal has been shown to have marked effects on the nutritive value of the resulting meal. Morrison *et al.* (8) demonstrated (Table I) that the nutritive value of the residual meal increased as the processing temperature was lowered. Opening the choke on the screw press (decreasing the pressure as the material goes through) resulted in the production of sunflower seed oil meal of superior nutritive value to that of meal processed under similar conditions with the choke in the regular position. Sunflower meal of equivalent nutritive value to high-quality, solvent-extracted soybean oil meal was produced by lowering the processing temperatures from 240°F. in the cooker and 260°F. in the conditioner, to 200°F. in the cooker and 220°F. in the conditioner and opening the choke on the screw press. As a result of reducing the temperatures and changing the choke setting, however, the oil content of the meal increased from 5.2 to 8.3%. This loss of oil would be at least partially compensated for by the improved quality of extracted oil and the increased nutritive value of the residual meal. The choice of operating conditions will depend on the relative economic value of the oil and

meal. It would appear that less drastic screw-press extraction followed by solvent extraction might offer a solution to complete, high-quality oil extraction coupled with the production of a top-quality sunflower seed oil meal.

The effect of excessive processing temperatures on the essential amino acid content of sunflower seed oil meal has been reported by Renner *et al.* (9). Less lysine, arginine, and tryptophan were found in a sunflower seed oil meal produced at conventional temperatures (at

TABLE I
EFFECTS OF PROCESSING VARIABLES ON THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF SCREW-PRESSED SUNFLOWER SEED OIL MEALS FOR POULTRY^a

Meal	Temperature		Choke ^b	Mois- ture (%)	Crude protein (N × 6.25) (%)	Oil (%)	Crude fiber (%)	Average weight of chicks in 24 days ^c (g.)
	Cooker (°F.)	Condi- tioner (°F.)						
Soybean oil meal, control								253.7
Sunflower seed oil meal	240	260		3.3	42.7	5.2	12.6	167.9
Sunflower seed oil meal	240	260	Open	4.1	39.3	7.6	13.5	235.6
Sunflower seed oil meal	200	220		4.3	38.3	7.2	14.2	240.7
Sunflower seed oil meal	200	220	Open	5.0	37.2	8.3	13.6	257.6

^a Data from A. B. Morrison, D. R. Clandinin, and A. R. Robblee, *Poultry Sci.* **32**, 492 (1953).

^b Denotes condition of choke on exit end of screw press. When it is open, less pressure and heat are developed.

^c Twenty White Leghorn cockerels per group.

240°F. in the cooker and 260°F. in the conditioner) than in sunflower seed oil meals processed at 200° to 220°F. in the cooker and 220° to 240°F. in the conditioner.

These findings are not in accord with the conclusion of Rombauts (10) that the nutritive characteristics of sunflower meal are unaffected by heat. It should be pointed out, however, that Rombauts was dealing with a high-quality sunflower flour which may not have contained a sufficiently high level of reducing sugars to effect a significant Maillard reaction (11, 12) under the moderate heat treatment employed (15 pounds steam pressure for 30 minutes). Had the heat treatment been more severe, Rombauts' conclusion might have been quite different. In

this regard Mitchell *et al.* (13) reported that when the nutritive value of the proteins of solvent-extracted sunflower seed meal processed at low temperature was compared with the same meal autoclaved at 20 pounds steam pressure for 30 minutes, the digestibility of the protein and its biological value, were significantly depressed by the heat treatment, with a total damage of 10.2% computed from the drop in net utilization. Alexander and Hill (14) have also reported damage to sunflower seed oil meal from excessive heat treatment. They found that screw-pressed meal had 30% less lysine than solvent-extracted meal.

TABLE II
PERCENTAGE COMPOSITION OF SUNFLOWER SEED OIL MEALS

Constituent	Type of meal							
	Strawy ^a		Hulled, ^a screw-pressed		Hulled, ^a solvent		Flour, ^b sol-	
	Average	Range	Average	Range	Average	Range	vent	Flour ^c
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Moisture	8.2	4-14	8.5	3-19	10	7-16	4.6	9.8
Crude protein (N × 6.25)	18.8	12-22	35.6	29-43	38.4	29-59	52.8	59.5
Fat	2.6	1.5-3.6	7.1	4-15	3.6	2-6	4.5	2.8
Nitrogen-free extract	22.1		26.5		26.6		27.6	16.8
Crude fiber	44.7	39-49	16.1	7-23	14.5	9-18	4.0	4.1
Ash	3.6	2.6-4.2	6.2	5-11	6.9	4-8	6.5	7.0

^a A. M. Leroy, A. François, H. Maitrejean, and B. Peronne, *Ann. agron.* [n.s.] **19**, 791 (1949).
^b H. H. Mitchell, T. S. Hamilton, and J. R. Beadles, *J. Nutrition* **29**, 13 (1945).
^c P. Rombauts, *Oléagineux* **6**, 203 (1951).

In addition, they showed that solvent-extracted meal after being heated in the autoclave for 30 minutes at 100°, 1 hour at 15 pounds steam pressure, or 2 hours at 15 pounds steam pressure contained 10%, 21%, and 51% less lysine, respectively, than the original meal. (For a general discussion of the effect of heat on vegetable proteins see Chapter 5.)

V. SUNFLOWER SEED OIL MEAL

1. Composition

a. General

The composition of sunflower seed oil meal varies according to the quality of the original seed and the method of processing. A wide variety of products are available on the market, ranging from low-quality, straw-like meals to high-quality flours. Table II (2, 15, 16) shows the

sort of variation in general composition that one might expect to encounter in various types of meals; for other data on this subject, see Rombauts (2). A reasonable standard for a high-quality dehulled meal would appear to be not over 12% moisture, not over 10% crude fiber, not less than 40% crude protein, and preferably less than 5% oil.

b. Amino Acids

Considerable data are available on amino acid distribution in the protein of sunflower seed oil meal. Selected data by both chemical

TABLE III
ESSENTIAL AMINO ACIDS IN PROTEIN OF SUNFLOWER SEED OIL MEAL
(Calculated as grams of amino acid per 16 g. of nitrogen)

Amino acid	Solvent-extracted, chemical ^{a,b}	Screw-pressed, microbiological ^{a,c}	
		Low temperature	High temperature
Lysine	3.8	3.3	2.8
Arginine	8.2	9.4	8.7
Tryptophan	1.3	1.0	1.0
Methionine	3.4	1.6	1.5
Histidine	1.7	2.1	2.1
Threonine	4.0	3.2	3.2
Leucine	6.2	6.1	5.9
Isoleucine	5.2	4.0	3.9
Valine	5.2	4.8	4.9
Phenylalanine	5.7	4.2	4.3

^a Refers to method used for analysis of amino acids.

^b R. J. Block and D. Bolling, *Arch. Biochem.* **6**, 277 (1945).

^c R. Renner, D. R. Clandinin, A. B. Morrison, and A. R. Robblee, *J. Nutrition* **50**, 487 (1953).

and microbiological methods of analysis are presented in Table III (9, 17). References to other data may be found in publications by Rombauts (2) and Block and Bolling (18). The values for methionine content obtained by microbiological methods, although in agreement with the value of 1.6% found by Dunn by the same method and reported by Block and Bolling (18), are not in agreement with the chemically determined value reported by Block and Bolling (17). The low methionine (microbiological) values might be accounted for by improper hydrolysis technique. In this regard Williams (19) has shown that in order to obtain valid methionine values for soybean oil meal by the microbiological technique, samples for assay should be hydrolyzed by refluxing rather than by heating in the autoclave which is the cus-

tomary hydrolysis procedure. This situation may also apply to sunflower seed oil meal.

Rombauts (2) has made a rather extensive comparison of the essential amino acid inadequacies of a large number of vegetable proteins, using egg protein as a standard, and has arrived at the conclusion that lysine is the limiting amino acid of sunflower protein. This confirms the biological studies of McGinnis *et al.* (20) and Slinger and co-workers (21) and tends to distinguish sunflower seed oil meal from soybean oil meal in which methionine is the limiting amino acid. Rombauts'

TABLE IV
CARBOHYDRATE CONTENT OF SUNFLOWER SEED OIL MEALS^a

	Flour	Hulled oil meal
	(%)	(%)
Crude fiber	4.1	7.8
Nitrogen-free extract	16.8	19
Formic acid index	5.7	13.5
Absorbable polysaccharides	14.6	13.2

^a Data from P. Rombauts, *Oléagineux* 6, 203 (1951).

comparison also brings out the fact that, of the vegetable proteins, sunflower shows the least deviation from the standard (egg protein), which leads him to conclude that sunflower protein is particularly suited to satisfy nitrogen requirements.

c. Carbohydrates

It has already been indicated (Table II) that the crude fiber content of sunflower seed oil meal varies according to the proportion of hulls removed prior to processing. According to Lemarchands (22) and Goldovskii and Bozhenko (23), sunflower seed oil meal does not contain the complex carbohydrates, starch, dextrin, or inulin, but does contain mono-, di-, and trisaccharides, some of which are reducing or which yield reducing sugars on hydrolysis.

Rombauts (2) presents data (Table IV) on two high-quality sunflower seed oil meals, comparing the carbohydrate content in terms of crude fiber and nitrogen-free extract against formic acid index [representing the total indigestible polysaccharides (24)] and the directly determined, easily hydrolyzed polysaccharides. The data support the contention that the samples contained more indigestible material than the crude fiber values indicate.

d. Minerals

Sunflower seed oil meal compares favorably with other vegetable protein feedstuffs as a source of calcium and phosphorus. Rombauts (2) reports 0.36% calcium, and 1.05% phosphorus in flour oil meal, and 0.49% calcium and 0.84% phosphorus in the hulled oil meal referred to above. The Illinois Agricultural Experiment Station (25) gives values for calcium and phosphorus of 0.57% and 0.58%, respectively, for sunflower seed meal. Sunflower meal contains relatively more calcium than phytin phosphate, and thus the assimilation of calcium is not impeded (26).

e. Vitamins

Solvent-extracted sunflower seed oil meal has been reported to be richer in B-complex vitamins than defatted wheat germ, corn germ, or defatted soybean oil meal (27). In this regard Rector *et al.* (28) presented data which indicated that sunflower seed meal contained approximately 150% more preformed nicotinic acid (184 γ /g.) than did the best grade of wheat germ analyzed (77 γ /g.) and was approximately 500% richer in this vitamin than corn germ (38 γ /g.) or solvent-extracted soybean meal (27 γ /g.). When one takes into account the "alkali-labile precursor" of nicotinic acid present in sunflower meal, which is biologically active for the chick (29), the total nicotinic acid value for sunflower meal [415 γ /g. (26) or 285 to 330 γ /g. (28)] is approximately the same, if not higher, than that of peanut meal [350 γ /g. (30)]. This is of considerable interest, since the latter has been rated as an outstanding source of nicotinic acid, when compared with other edible oilseed and cereal products (31). The pantothenic acid content of sunflower seed meal (43 γ /g.) was found by Rector *et al.* (28) to be similar to that of solvent-extracted soybean meal (43 γ /g.) and 60% greater than that of soybean meal produced by the screw-press process (26 γ /g.). Corn germ (25 γ /g.) and wheat germ (30 γ /g.) were found to have less pantothenic acid than sunflower seed meal. Literature values for thiamine, riboflavin, nicotinic acid, and pantothenic acid are: thiamine, 36 γ /g. (25); riboflavin, 3.1 to 4.5 γ /g. (2), 5 γ /g. (25), 3.1 γ /g. (32); niacin, 90 to 105 γ /g. (2), 300 γ /g. (25), 415 γ /g. (26), 285 to 330 γ /g. (28); and pantothenic acid, 33 to 58 γ /g. (28).

2. Nutritive Value

Using the nitrogen-balance method and rats as experimental animals, Mitchell *et al.* (16) found that the protein of low-temperature, solvent-extracted sunflower seed flour was highly digestible (94.3%)

and possessed a biological value of 64.5%. Bricker and Smith (33), using human subjects and a sunflower flour processed in a manner similar to that used by Mitchell *et al.* found that the protein was $89.9 \pm 0.86\%$ digestible and had a biological value of $61.2 \pm 1.71\%$. Rombauts (2, 10) reported that the protein of sunflower flour had an apparent digestibility coefficient of 88% and a biological value of 64%. It would appear, therefore, that high-quality sunflower flour protein is superior to most (equal to soybean oil meal protein) vegetable proteins from the point of view of digestibility (2) and is quite comparable to other vegetable proteins in so far as biological value is concerned, being practically equivalent to properly processed soybean oil meal in this respect (10). It should be pointed out, however, that had poor-quality (high-fiber) sunflower seed oil meals been used in the above-mentioned studies the digestion coefficients would have been lower and perhaps would have more nearly approached the figure of 71% reported by Jacquot (34) and that of 55 to 71% reported by Matet and co-workers (35).

On the subject of nitrogen retention from sunflower flour by growing rats, Rombauts (10) reported a retention coefficient of 46 compared to one of 52.5 for casein. These values agree with the respective values of 46 and 50 found by Matet *et al.* (35) under similar levels of protein feeding.

Rombauts (2) has also reported that the polysaccharides of sunflower flour were 95.7% digestible by the rat. He found that the digestibility of phosphorus was low, which he attributed to the fact that an appreciable proportion of the phosphorus in sunflower seed meal occurs in the phytin form. He pointed out, however, that the animals stayed in positive phosphorus balance because of high retention. In so far as calcium retention was concerned, Rombauts' data showed that the animals remained in positive balance and hence led him to the conclusion that sunflower seed meal satisfied the body requirement for this element.

Fraps (36) reported a productive energy value of 110 therms per hundred pounds of sunflower seed oil meal containing 34.8% protein, 18.3% fat, 10.9% crude fiber, and 21.8% nitrogen-free extract. A soybean oil meal containing 43.9% protein, 5.5% fat, 5.8% crude fiber, and 29.9% nitrogen-free extract was shown to have a productive energy value of 64.9 therms per hundred pounds. It should be pointed out, however, that had the sunflower seed oil meal, studied by Fraps, contained the usual amount of fat (4%) found in commercial sunflower seed oil meal, the productive energy value reported for sunflower seed oil meal would have more nearly approached that reported for soybean oil meal.

VI. USES OF SUNFLOWER SEED OIL MEAL

The nutritive value of sunflower seed oil meal as a feedstuff for poultry and other livestock, as previously indicated, is materially affected by the quality of the original sunflower seed, the completeness of the hulling operation, and the conditions to which the decorticated meats are subjected during the extraction of the sunflower oil. The chemical and nutritive data presented thus far would indicate that high-quality sunflower seed oil meal should be an excellent protein supplement for poultry and other livestock. Indeed, high-quality meals are used extensively in poultry and swine feeds in place of soybean oil meal and other vegetable protein supplements, and lower-quality meals (high fiber meals) and ground sunflower heads are used for feeding cattle. Specific work that has been reported on the use of sunflower seed oil meal in human, poultry, swine, and cattle nutrition is reviewed in the sections that follow.

1. Human Nutrition

Sunflower flour, because of its high protein content and high digestibility, is considered to be a suitable food for use in infant nutrition (37). It is not, however, recommended as a milk substitute (34). The Home Economics Department of the University of Illinois (25) tested sunflower seed flour additions to plain, spice, and chocolate cakes and in griddle cakes, muffins, and yeast rolls, in each case replacing a part of the white patent flour with sunflower seed flour. Many of the products containing 10% of sunflower seed flour were reported as being delicious. It could, not, however, be used successfully in light-colored products, as the addition of sunflower seed flour caused a gray cast to develop. The use of sunflower seed flour increased the protein and vitamin content of the baked products. (See also discussion in Chapter 9.)

2. Poultry Nutrition

Grau and Almquist (38) found that sunflower seed oil meal when included in a semi-synthetic chick starter ration to supply 20% protein was a complete source of essential amino acids required by the chick for growth. McGinnis *et al.* (20), Slinger and co-workers (21), and Alexander and Hill (14), on the other hand, have shown that practical diets containing commercial sunflower seed oil meal as a source of supplementary protein are apt to be deficient in lysine. That such need not necessarily be so is supported by the fact that solvent-extracted (low-temperature) sunflower seed oil meal is much higher in lysine

than screw-pressed (high-temperature) sunflower seed oil meal (14), and by the chick feeding experiment reported by Ewing (39) in which a high-quality South American sunflower seed oil meal was not improved by lysine supplementation.

Various workers have reported experiments dealing with the use of commercial sunflower seed oil meal in poultry rations to replace other protein supplements. Pettit *et al.* (40) found that sunflower seed oil meal could replace all the meat meal (10.5%) in the chick starter ration employed. O'Neil (41) found that sunflower seed oil meal was a satisfactory substitute in chick starter rations when replacing not more than one-third of the total animal protein mixture. Kondra and Hodgson (42) stated that 2% sunflower seed oil meal in a chick starter ration containing 7½% meat meal, 2% fish meal, and 2% skim milk powder produced normal growth in chicks up to 7 weeks of age, whereas 10% sunflower seed oil meal combined with 3¾% meat meal in the absence of fish meal and skim milk powder resulted in decidedly slower-than-normal growth rate to 7 weeks of age. On the basis of results obtained in feeding trials, Nikolaiczuk *et al.* (43) concluded that sunflower seed oil meal provides a nutritionally incomplete protein for chick growth but that it is superior to soybean oil meal as a source of protein for the chick. These workers also observed that sunflower seed oil meal has high complementary value.

In an extensive series of experiments involving processing of sunflower seed oil meal at low temperature (processed in the cooker for 30 minutes at 200°F. and in the conditioner for 3 minutes at 220°F.) and at regular temperature (processed for similar periods of time in the cooker at 240°F. and in the conditioner at 260°F.) Morrison *et al.* (44) showed that the "low-temperature" meal satisfactorily replaced two-thirds of the fish meal and meat meal mixture, all the meat meal, or two-thirds of the soybean oil meal in the practical starter rations used. "Regular-temperature" sunflower seed oil meal could replace one-third of the fish meal and meat meal mixture, or two-thirds of the soybean oil meal in the starter rations. When 2½% fish meal was included in the starter ration, "regular-temperature" sunflower seed oil meal satisfactorily replaced one-third of the meat meal in the ration. When meat meal was the only protein supplement in the starter, "regular-temperature" sunflower seed oil meal replaced all the meat meal in the ration.

Gartley *et al.* (45) reported that under the conditions of their experiment sunflower seed oil meal was a satisfactory protein supplement for the growth of turkey poults when used at the level of 21% or less in a 28% protein ration for the first 4 weeks. From 4 to 8 weeks, use of as much as 16.5% sunflower seed oil meal resulted in satisfactory growth on a 26% protein mash.

Tabakoff (46) recommended the use of up to 25% sunflower seed oil meal in the ration of layers. More recently Pettit *et al.* (40) reported that sunflower seed oil meal can adequately replace part or all the soybean oil meal, or half of the meat meal, or half of the fish meal in the laying and breeding rations used. It was also found that all the

soybean oil meal plus half of the meat meal could be replaced with sunflower seed oil meal in their ration without any serious decrease in egg production or hatchability.

3. Swine Nutrition

Weaver (47) reported that sunflower seed oil meal compared favorably with tankage as a protein supplement for dry-lot fattening of hogs weighing over 100 pounds. Krider *et al.* (48) showed that sunflower seed oil meal can be used as part of a protein-vitamin supplement for dry-lot hog feeding to replace a part of the soybean oil meal and/or meat meal. When 10% meat meal was replaced, gains were unaffected, but when both meat meal and soybean oil meal were replaced, the rate of gain was significantly decreased. Tangl *et al.* (49) have shown that the protein, fat, and nitrogen-free extract of sunflower seed oil meal are well digested by the pig. Sunflower seed oil meals that varied in protein content from 31.4 to 49.1% and in fat from 1.5 to 15.6% were fed to weanling pigs weighing about 26 pounds. Digestion values for protein, fat, and nitrogen-free extract ranged from 77 to 81%, 28 to 63%, and 87 to 92%, respectively. Pearson *et al.* (50) found that hexane-extracted sunflower seed oil meal containing 45% protein was inferior to both peanut meal and soybean oil meal as a source of protein for the pig. The sunflower meal used was apparently low in lysine, as supplementation with this amino acid improved a ration of corn-sunflower seed oil meal.

4. Cattle Nutrition

Sunflower seed oil meal is said to be a popular feed in Europe for all classes of livestock, especially for dairy cows. In the latter regard, Popov (51) reported on the uses of various oilseed cakes as feed for cattle. He stressed the desirable characteristics (high protein content and ease of feeding) of linseed oil cake and sunflower oil meals.

Pearson *et al.* (50) reported on two experiments involving a total of 22 steers in which sunflower seed meal was compared with cottonseed meal as a source of supplementary protein. Results obtained indicated that the two protein supplements were about equal for fattening steers from the standpoint of average daily gain, feed requirement per unit of gain, dressing percentage, and carcass grade.

VII. BY-PRODUCTS

1. Oil

To the feeder, sunflower oil might be looked on as a by-product obtained in the processing of sunflower seed for sunflower seed oil meal.

To the processor, however, sunflower oil is the product and sunflower seed oil meal the by-product. Sunflower oil is similar in composition to corn oil although it differs from corn oil in that it contains more unsaturated fatty acids. It is classed as a semi-drying oil. In addition it possesses the desirable characteristics of not becoming rancid, even on long exposure to air, and of not separating out on long refrigeration (52).

2. Hulls

The husks obtained in the decortication of sunflower seed prior to extraction of oil may be used as bedding for poultry and livestock. In Canada, however, most of the hulls are molded under high pressure into logs which are suitable for burning in fireplaces.

3. Other By-Products

Rudorf (6) has shown that the heads (receptacles) of the sunflower plant may be used as a source of pectin and that the stalks, which contain 40 to 48% α -cellulose, by special chemical treatment may be used in the production of paper and textiles.

Smith and Johnsen (53) have developed a method for the isolation of sunflower protein. Petrov and Grinberg (54) have shown that sunflower protein may be used for the production of plastic materials, and Petrov and Dimakov (55) have described its use for the production of detergents. (See also Chapter 10.)

VIII. TRENDS

The world harvest of sunflower seed in 1953-54 was reported (1) as 4.7 million tons, compared with the record crop of 5.0 million tons in 1950-51; in 1955 the figure was 5.6 million tons (56). In 1953-54 sunflower seed production in the Soviet Union was reported to have shown a further increase, but the 1953-54 crop in Argentina, the major non-Communist producer, was the smallest for many years, amounting to only 380,000 tons, compared with more than 1.1 million tons of seed in 1950-51. This decline in sunflower seed production in Argentina has been widely attributed to the relatively low official price paid to farmers in Argentina for sunflower seed and to unfavorable weather conditions for sunflower production. In 1954 and 1955, to encourage sunflower production, the Argentine Government announced substantial advances in the price that would be paid to farmers for sunflower seed. As expected, these increases in price, which have practically doubled the paying price for sunflower seed, stimulated production in 1956 to a level of 831,000 tons (56).

In spite of the paucity of information on the use of sunflower seed oil meal in swine and cattle rations its use in poultry and livestock feeds is becoming more generally accepted as being sound practice. Once processors recognize the importance of paying attention to more complete decortication of sunflower seed before extraction and to techniques in the processing of the decorticated sunflower meals that will not damage the protein residues, there does not appear to be any nutritional reason why sunflower seed oil meal should not take its rightful place near the top of the list of protein-rich feedstuffs for inclusion in poultry and livestock rations.

IX. SUMMARY

Large quantities of sunflower seed are produced in the U.S.S.R., the Balkan countries, and South America. Relatively insignificant quantities of this protein-rich seed are produced in North America. As more suitable types of sunflower seed are developed, production of this crop may expand.

Processing methods employed in the extraction of oil from sunflower seed have marked effects on the nutritive value of the residual meals, particularly for non-ruminant animals. In this regard, a maximum amount of hull should be removed from the seed prior to the extraction of the oil, and the decorticated meals should be subjected to a minimum of heat during the extraction process if high-quality sunflower seed oil meals are to be obtained.

In general, as a feed for poultry and livestock, high-quality sunflower seed oil meal may be used interchangeably with soybean oil meal. Lower-quality sunflower seed oil meals, however, should be used in rations for non-ruminants with considerable discretion.

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CHAPTER 20

RAPESEED, MUSTARD-SEED, AND POPPY-SEED MEALS

B. C. CHRISTIAN

I. RAPESEED MEAL

1. Introduction

Rape oil has been used for many centuries for edible purposes and as an illuminant, often under the name of Colza oil. During both World Wars rape oil became of major importance as a constituent of human food. Rape and mustard are members of the *Brassica* genus, and the seeds are an important source of oil, of which they contain about 40%; usually no distinction is made in trade and commercial statistics between the two as an oilseed crop.

Rapeseeds and mustard seeds are characterized by the presence of glucosides which are readily hydrolyzed under certain conditions by enzymes in the seeds to produce pungent "mustard oils" and toxic compounds. This property is utilized in the preparation of condiments from some varieties of mustard, but rape and the varieties of mustard grown as an oilseed contain the least amounts of the obnoxious glucosides.

Rapeseed meal contains about 35% protein and is a valuable potential animal feedstuff, but there is a hazard in its use unless care is taken to avoid enzymatic breakdown of the glucosides during oil processing. Some authorities think that the only sure way of avoiding the ill effects of the meal is to limit the amount fed to 10% of the ration. There is need for more detailed study of the relation between processing of the meal and its value for animal feeding.

2. Production and Trade

The cultivation of rape for oilseed production is mainly carried out in the temperate and warm temperate zones of Asia and Europe where summer and winter varieties are both grown. Rape was once the most

important oilseed crop in Western Europe, but there was a sharp decline in production after 1860, when mineral oils replaced rape oil for lubrication and illumination. Interest in this crop in the present century has been confined mainly to periods of acute shortages of vegetable oils.

Very little rapeseed was produced in Holland before 1939, and the seeds were mainly imported from India. During World War II the growing of rape was heavily subsidized, and a good crop (80,000 tons) was obtained in 1943, but in 1944 the crop failed, owing to the well-known sensitivity of rape to weather and insect attack. Since then, appreciable quantities of rape have not been grown in Holland.

The growing of rape began in Sweden in 1941, and since then the area devoted to rape has been increased, but cultural difficulties were encountered, as in Holland, and the development of weather-resistant strains of rape is being investigated.

In the United States and Canada, rape is grown mainly as a forage crop, for which special varieties are used. Shortages of oil during World War II, however, stimulated work on the production of rapeseed for oil in Canada. The 1954 Canadian crop was the heaviest since 1948; in 1955 34,500 tons were reported, and preliminary estimates indicate 135,000 tons grown in 1956.

The loss of Manchurian soybean supplies and the shortage of foreign exchange have stimulated rapeseed cultivation in Japan. A temporary fall in prices led to a sharp decline in planted areas and production in 1954, but preliminary figures indicated a recovery in sown areas to 540,000 acres in 1955, with a total yield of some 285,000 tons, a crop equal to the record 1953 level. The 1956 crop is estimated at 320,000 tons.

India and Pakistan are the only countries in the British Commonwealth producing large quantities of rapeseed. It is by far the most important source of edible oil in Pakistan, where cultivation has increased since World War II. The Indian agricultural plan envisages an average crop of nearly a million tons during the second half of the present decade.

World rapeseed production in 1954-55 was well below the record 1952-53 level, despite increased output in China. Production figures are given in Table I (1). Total exports of seed in 1954 [approximately 50,000 long tons (1)] were the smallest since 1949, reflecting the sharp decline in Swedish shipments and the virtual cessation of supplies from China, Eastern Europe, and France. Exports of oil in 1954 were also the smallest during the post-war period under review, and less than one-third of the record 1953 quantities. Swedish shipments were halved and there were only small supplies from other sources.

TABLE I
PRODUCTION OF RAPESEED^a

Country	1938 and 1938-39	1949 and 1949-50	1950 and 1950-51	1951 and 1951-52	1952 and 1952-53	1953 and 1953-54	1954 and 1954-55
Thousand long tons							
Argentina	23	3	3	3	1	1	1
Austria	2	4	3	3	9	9	6
Belgium	—	9	3	5	4	2	1
Canada	—	8	—	3	7	12	13
China	(2400)	3010	(3000)	(2550)	(3100)	(2850)	(3000)
Denmark	—	1	2	9	23	19	11
Finland	—	—	1	7	18	20	12
France	10	140	125	161	221	92	87
Western Germany	54	138	79	85	55	31	15
Eastern Germany	72	(110)	—	—	—	—	—
India ^b	700	793	750	928	844	858	962
Italy	1	11	11	12	13	11	7
Japan	117	38	117	176	278	284	216
Netherlands	6	58	44	21	13	10	16
Pakistan ^b	223	236	278	302	228	272	324
Poland	71	90	114	(110)	(85)	(78)	(100)
Sweden	—	114	169	206	166	79	153
Switzerland	—	4	4	6	7	4	6
Yugoslavia	9	4	5	8	5	12	3
Others	115 ^c	45	55	60	60	55	60
Total ^d	3803	4816	4864	4755	5230	4799	5093
Total excluding China	1403	1806	1864	2205	2130	1949	2093

^a Commonwealth Economic Committee, "Vegetable Oils and Oilseeds." H. M. Stationery Office, London, 1956.

^b Rape and mustard.

^c Roumania 52, Bulgaria 21.

^d Includes rough estimates (in parentheses) where other figures are not available. The Soviet Union is excluded from both "others" and total.

3. Botanical Information

The cultivated varieties of rape are mainly variations of *Brassica napus* (rape) or *Brassica campestris* (field mustard). Both may be grown as annuals or biennials, depending on variety and time of sowing.

Brassica napus thrives in rich soil in a cool, moist climate; it is closely related to the rutabaga or Swede turnip (*B. napobrassica*) and resembles it except that the taproot of rape does not form a tuber like that of rutabaga. The plants grow 2 to 3 feet high and have thick succulent stems and leaves; the leaves have a bluish glaucous color like

cabbage leaves. *B. campestris* differs from *B. napus* in having thinner and less succulent leaves of a greener, less glaucous color. It is more like the turnip (*B. rapa*) in these respects.

Summer rape varieties are sown early and mature in the same year; winter varieties are sown in the autumn and mature in the following year. Rape grown in the United States, England, and Europe mostly has dark-colored seeds; much of the Asiatic rape is lighter in color, some being almost white.

Leafy varieties of rape (e.g., Dwarf Essex) are grown in the United States and Canada as a forage crop, and in Europe and elsewhere less leafy varieties are grown for oilseed production. Varieties of both *B. napus* and *B. campestris* are grown in Europe, and commercially no distinction is made between the oils from the different varieties.

Rapeseed has no endosperm and consists entirely of embryo and seed coat. The oil content varies with variety and locality, but 40% oil is a typical figure. Rapeseed oil is considered as a semi-drying oil; it contains about 40 to 50% erucic acid (13-docosenoic acid).

4. Composition of Meal

The composition of rapeseed meal varies with the method of oil extraction. Typical meals and cakes contain about 35% crude protein; further details are given in Table II.

There are no published data on the vitamin content of rapeseed meal, but Bell (2), in his excellent review, suggests that it is unlikely to differ greatly from that of other oilseed meals. Calcium and phosphorus contents have been reported as 0.51 to 0.61% and 0.80 to 0.84%, respectively (3), and the meal is particularly rich in phytin phosphorus (4). The determination and content of phosphatides is described by Rewald (5).

The amino acid content of the protein in rapeseed meal is shown in Table III (6, 7). The figures in the last column show the amino acid requirements of chicks, expressed as a percentage of the protein content of the ration, with 20% protein in the ration. (See also Table III, page 24.) Nutritional experiments suggest that rapeseed meal can supply adequate levels of all the essential amino acids for chicks, with the possible exception of lysine (see also references 8-10).

5. Toxicity

a. Nature of Toxic Factor(s)

Numerous reports have indicated that rapeseed meal contains a toxic factor, but findings differ as to its nature and significance, and some suggest that more than one factor is involved (11-13).

TABLE II
COMPOSITION AND DIGESTIBILITY OF RAPESEED, MUSTARD-SEED AND POPPY-SEED MEALS AND CAKES

Meal or cake	Type of processing	Moisture	Ash	Crude protein ^a	Digestible crude protein	Nitro-gen-free extract	Crude fiber	Crude oil ^b	Total digestible nutrients	Starch equivalent	References
		%	%	%	%	%	%	%	%	%	
Rapeseed	Solvent extraction	—	7.3	35.1	—	31.3	13.4	2.0	—	—	(62)
	Solvent extraction ^c	10.7	7.3	36.9	30.7	32.7	9.3	3.1	60.3	53.9	(29)
	Press	11.6	6.9	30.9	26.5	30.0	9.0	11.6	73.5	—	(63)
	Solvent extraction	11.4	7.0	34.2	—	34.7	11.4	1.3	—	—	(64)
	Press	11.3	7.4	34.7	28.5	30.3	9.7	6.6	63.3	—	(63)
Mustard seed	Press ^c	8.6	12.4	35.5	29.4	25.6	8.3	9.6	58.2	59.5	(29)
	Solvent extraction	—	7.3	42.8	—	29.2	14.1	0.2	—	—	(62)
	Solvent extraction ^c	12.0	6.6	22.8	—	40.6	16.0	2.0	—	—	(29)
Poppy seed	Press ^c	12.0	5.0	18.0	—	40.0	17.5	7.5	—	—	(29)
	Solvent extraction	—	13.5	36.0	—	21.3	17.0	0.7	—	—	(62)
	Solvent extraction	9.5	11.5	36.1	—	24.0	16.7	2.3	—	—	(58)
	Press ^c	9.5	13.8	36.9	29.2	21.7	8.3	9.8	—	62.3	(29)
	Press (range)	4.3-17.7	10.1-14.5	24.4-41.6	—	8.5-29.6	4.9-22.8	3.8-17.1	—	—	(65)
	Press (French)	10.1	13.5	37.3	—	14.4	12.4	8.1	—	—	(65)
	Press (French)	11.2	14.9	32.0	—	21.3	11.1	5.7	—	—	(65)
	Press (German)	8.5	13.3	40.5	—	15.8	9.2	8.6	—	—	(65)
	Press (German)	8.5	10.8	38.4	—	16.9	8.0	13.4	—	—	(65)

^a Nitrogen $\times 6.25$.^b Ether extract.^c Considered a typical composition.

In 1901 (14) crotonyl isothiocyanate was identified in the essential oil fraction of rapeseed. Viehoever *et al.* (15) found crotonyl and allyl isothiocyanates in rape and mustard seed. In 1949 (16, 17) a goitrogenic substance was isolated from rapeseed, which proved to be L-5-vinyl-2-thioöxazolidone (see also reference 18). At about the same time the glucosides sinigrin and gluconapin were also identified (13); these substances are precursors of crotonyl and allyl isothiocyanates and possibly have some relationship to thioöxazolidone (16, 19). The

TABLE III
AMINO ACID CONTENT OF RAPESEED PROTEIN^a

Amino acid	Roche and Michel (6)	Agren (7)	Bell (2)	Chick requirements ^b
	%	%	%	
Arginine	6.6	5.6	7.2	6.0
Histidine	—	2.6	—	0.75
Isoleucine	—	3.7	4.5	3.0
Leucine	6.9	5.7	8.7	7.0
Lysine	—	3.5	5.4	4.5
Methionine	—	1.1	5.3	2.25
Phenylalanine	1.9	4.0	—	4.5
Tryptophan	1.2	2.0	—	1.0
Valine	4.2	5.7	6.5	4.0
Threonine	3.3	3.8 ^c	4.8	2.25
Cystine	2.4	1.7	1.9	—
Tyrosine	—	2.3	6.6	—
Alanine	3.2	1.9	5.0	—

^a Calculated as grams per 16 g. of nitrogen. Taken from Bell (2).
^b Percentage of protein content based on 20% protein in the ration. See Table III, Chapter 2.

isothiocyanate contents of solvent-extracted rapeseed meal vary from 0.3 to 1.4% (2) [see also Schwarze (20)].
The response obtained on feeding iodide in rations containing rapeseed meal led to the conclusion that the toxic factor must be a thiocyanate (21). Since then, studies with iodide treatment have supplied most of the information on the mechanism of the action. Iodide was only partially effective against the rapeseed factor when fed to rats; it reduced thyroid gland enlargement, but the hyperplastic state persisted (22). Kratzer (8) reported that the enlarged thyroid in chicks, attributed to rapeseed meal, was not countered by feeding potassium iodide. Greater success was achieved with iodinated casein (Protamone), which reduced thyroid enlargement in turkey poults to a

considerable degree at levels of 0.022% of the total diet (8-10); however, Nordfeldt *et al.* (23) obtained only partial reduction of the toxic effect by using iodinated casein in pig rations.

Other reports (22, 24) state that synthetic thyroxine inhibits thyroid hyperplasia in rats when added to the ration at levels of 3 mg. per 100 g. of body weight and also is an efficient antithyroid factor in turkey rations.

b. Detoxification

Water extraction *in vacuo* (23) at 100° removed most of the toxicity; other reports recommended hot-water extraction (25, 26), but Frolich (18) claimed that cold water gave satisfactory results. Kratzer (8) had little success with any water treatment. Steaming is said to destroy gluconapin, and, in one instance this treatment reduced the content of sulfurated essential oils in two samples from 0.39 and 0.25%, respectively, to 0.014% (27). Bell (2) found that the reincorporation of aqueous extracts of rapeseed meal after soaking, steam heating, or mild acid hydrolysis resulted in more serious growth depression than that which resulted from the use of the untreated meal. According to Allen and Dow (25), dry heating has little effect in reducing goitrogenic activity, but Frolich (18) claimed that dry heating at 150° inactivated the toxic principle. Several reports describe solvent treatments; for instance, the toxic glucosides can be removed with 70% ethyl alcohol (13, 18, 28).

Bell (2) concludes that the only satisfactory method of counteracting the total effect of the toxic factor in rapeseed is to limit the use of the meal.

6. Processing

First-grade edible oil was formerly produced by cold pressing, but nowadays a greater yield is obtained by hot pressing and solvent extraction.

The formation of mustard oil in the cake by enzyme action is influenced by the moisture content of the seed, the fineness of grinding, the drying and pressing temperatures, and the length of time under the various conditions. An important part is played by the enzyme myrosine, which catalyzes the decomposition of sinigrin and sinalbin, with the formation of the essential mustard oils. These, together with the products of other enzymatic reactions, have a marked influence on the refining and hydrogenating properties of the oil, and on the toxicity of the meal. The inactivation of the enzymes before processing is therefore a very important objective. This may be achieved during the heat-

ing and drying process before the seed is pressed; the degree of inactivation will depend chiefly on the moisture content of the seeds.

Rapeseed is first ground by passing through either five-high Anglo-American rolls or four-pair-high sets of rolls, the latter having the top and second pairs of rolls with flutes at $\frac{1}{2}$ -inch and $\frac{3}{16}$ -inch pitch, respectively, driven at differential speeds, and the third pair with $\frac{3}{4}$ -inch pitch scratch flutes and bottom plain rolls driven at equal speeds. Although this treatment increases the possibility of formation of mustard oil, the conditions will not be favorable if the moisture content of the seed is low and the grinding not too fine.

The ground seed is cooked in a steam-jacketed kettle with little or no open steam. Provided that the initial moisture content of the seed does not exceed 12%, it is permissible and even desirable to raise the seed temperature to 95° in order to inactivate the myrosine and prevent enzymatic formation of mustard oils.

During the cooking, as the seed passes through the temperature range 30° to 60°, conditions are favorable for the action of myrosine. The formation of mustard oil is retarded, however, at lower moisture contents; it is restricted at 8% moisture content but is nevertheless possible. Not until the seed has attained a temperature of 80° is the myrosine inactivated.

Seeds of high initial moisture content are heated at a moderate temperature until their moisture content is reduced to 12%, and subsequently at 95° to 8% moisture. In this way inactivation of the enzymes is attained without the hardening and bleaching capacities of the oil being significantly impaired. The most favorable initial moisture content of seeds for processing is 8%. The cooked seed is passed through screw presses either once or twice, reducing the oil in the cake to about 23% with one pressing and to about 12% with double pressing. The moisture content of the cake is about 8%.

The next stage is solvent extraction of the cake. The press cake is broken down in two-pair-high cracking rolls, heated in rotary dryers to about 75°, and then passed through flaking rolls. The flakes are solvent-extracted in a batch-type plant. Petroleum (boiling point 70° to 90°) is pumped through the flakes, entering the top of the extractor at 70° to 75°, and reducing the oil content to about 0.5%. The meal is desolventized in 25 to 30 minutes with steam at 25 to 28 p.s.i. and 182° to 240°, admitted at the bottom of the extractor. It is discharged with a moisture content of about 14% and broken down in a nutter and flextooth crusher before passing through a rotary steam dryer, cooler, and grinder for bagging.

The contents of digestible material in rapeseed cake and extracted meal are shown in Table II. The content of total digestible matter

of rapeseed meal is about 10% less than that of soybean and linseed meals; this is attributed to the relative indigestibility of the nitrogen-free extract and the crude fiber. The average protein digestibility is comparable to that of other oilseed meals (82 to 86%) (29).

7. Uses

a. Cattle

Feeding trials with cattle and sheep have indicated that ruminants are less susceptible than other classes of livestock to the toxic effects of rapeseed meal. Early work (30-32) showed that 2 to 3 kg. of dry meal could be fed daily to dairy cows, but it was refused when moistened with water. In nutritive value it was equivalent to other oilmeals, and the only ill effect was a tendency to lower the milk fat content. Denisov recommended not more than 2.5 kg. per head for dairy cattle, but as the meal was not readily eaten it was necessary to mix it with more palatable feeds (33). Richter (34), Cuttano and Marracino (35), and Seale (36) found that rapeseed meal increased milk yield, in one case by 4%, and had no significant effect on the fat content. Burkitt *et al.* (3) fed up to 2 lb. of rapeseed daily to beef cattle without ill effect, and this level was also suitable for calves, yearlings, and cows in calf.

Current opinion is that not more than 2 lb. per head per day should be fed, and special caution should be exercised in the case of young and pregnant animals (37).

b. Sheep

Limited amounts of rapeseed meal have been fed to weaned lambs and pregnant ewes with no detrimental effects, although palatability has been a problem. Rapeseed meal compared favorably with linseed meal except in palatability, and there was no thyroid enlargement. Rapeseed cake was suitable for 10- to 11-month wethers when fed at levels up to 200 g. daily (38).

c. Pigs

Particular care must be taken when feeding rapeseed meal to pigs. Under certain conditions (39), up to 400 g. per head per day was satisfactory for suckling sows and 200 g. per head per day for young pigs, but the meal was not suitable for fattening pigs. The mustard oil content had no effect on breeding sows, and digestive disturbances could be alleviated by feeding charcoal (40). Seale (see reference (2)) used 20% rapeseed meal in the ration and found no adverse effect on growth rate, feed efficiency, or carcass quality, although there was histological evidence of thyroid disturbance. At 10 and 20% levels, growth depres-

sion and enlargement of the liver, kidney, and thyroid were reported. Cook found that it was practicable to substitute half the meat meal in a standard ration by rapeseed meal (41).

The general opinion is that it is permissible to use rapeseed meal to the extent of about one-third of the protein supplement in the rations of growing and finishing market hogs. No recommendations can yet be made for other classes of pigs.

d. Poultry

The use of large quantities of rapeseed meal in poultry rations may result in goitrogenesis and growth inhibition. Such effects have been reported on feeding laying hens at levels of 15% (42), growing chicks at 20% (8, 43, 44), and turkey poults at 25% (45). A 10% replacement of soybean meal by rapeseed meal (46) lowered the growth rate of chicks by 11% and the feed efficiency by 27%, but 2.5 or 5% levels gave satisfactory growth. Adverse effects on growth and egg production in chicks and laying hens were noted (47) when more than 5% rapeseed meal was fed. Blakely (24) suggested that the depressed growth rate in turkeys on 7% rapeseed meal was due not to a protein deficiency but to the presence of toxic agents.

The use of Protamone has already been discussed; for poultry less than 36 g. per 100 lb. of feed gave adequate protection against the toxic effect of the meal (10). Conflicting evidence exists concerning the value of iodide in combatting the toxic effects.

In contrast to the results already mentioned, Kondra and Hodgson (48) found rapeseed meal to be a complete protein food for chick starter rations and satisfactory as a replacement for sunflower seed meal. Allen and Dow (25) suggested that rapeseed meal should be used in starter rations only after hot-water extraction or in conjunction with iodide. When fed at levels up to 25% of the total ration, rapeseed meal improved feed efficiency and body weight, the effect being particularly noticeable in the lower range.

Several authorities claim that rapeseed meal in the diet reduces the incidence of perosis and dermatitis (9, 10), although it may be responsible for lysine deficiencies, which might explain the depression of growth rate and poor feather pigmentation in poults (8, 9). There are no reports on palatability problems associated with feeding this meal to poultry.

The current view is that rapeseed meal should not be used in starter rations but should be reserved for fattening birds for which the maximum desirable level is about 10% of the total ration; this level may be too high for turkeys (2, 8, 24, 37, 49).

II. MUSTARD-SEED MEAL

1. General Information

As pointed out earlier, no differentiation is made between rape and mustard as an oilseed crop. The identity of mustard seed has slight commercial significance, and only a little information on mustard-seed meal itself is available.

Mustard and rape are grown as a mixed crop for oilseed production; the main sources of supply are the United States, Canada, Western Europe, Southern Italy, and the U.S.S.R. Mustard-seed production in the United States averaged 21,500 tons per year for 1941–45. Statistics are not available for other areas; Table I gives some figures in combination with rape.

Mustard belongs to the genus *Sinapis* and to the same family (*Cruciferae*) as rape. Eight varieties of mustard are known; among them are white and black mustard (*S. alba* and *S. nigra*), which are used for oilseed production. Other varieties are usually grown for condiment manufacture. The plant is quick-growing and reaches a height of 1 to 4 feet by three or four months after sowing. The flower head is similar to that of wild turnip and is composed of small lemon-yellow florets; the leaves are small, dark green, and hairy. The yellow seeds of white mustard are contained in hairy rounded pods; black mustard seeds are produced in smooth erect pods.

There is little information on the composition of mustard-seed meal. A typical cake contains 18% protein, and a meal about 23% protein. Further details are shown in Table II. No information is available on the amino acid composition of mustard-seed protein.

Mustard-seed meal, like rapeseed meal, contains glucosides which give rise to pungent toxic mustard oils on enzymatic hydrolysis. The concentration of these substances is higher than in rapeseed meal, and prolonged steaming is required before the meal can be used as a protein supplement for livestock and poultry rations (50).

Very little mustard seed is processed for oil, and processing details are not available. Mustard seed and rapeseed often occur as mixtures in the trade and are processed as rapeseed, previously described. Mustard seed oil also contains 40 to 50% erucic acid and is classed as a semi-drying oil.

2. Uses

There is very little information on the digestibility of mustard-seed cake; the digestibility coefficient of the true protein is quoted as 83% (51).

a. Dairy Cattle

In 1943 a trial was carried out with 30 milking cows which were fed 3 kg. of extracted mustard-seed meal per head per day as part of their concentrate ration; the meal had been steamed for 2 hours and contained 43% crude protein. No adverse effects were observed in the cows or the milk. It was recommended that the meal should be fed dry and preferably mixed with other concentrates at 1.5 kg. per day (52, 53).

The feeding of Chinese mustard cake (600 g. per head per day) to dairy cattle increased the iodine value of the butter fat from 24 to 30. The taste and odor of the milk were unaffected (54).

b. Poultry

Chick rations containing 8% animal protein and 2.8, 5.6, 8.4, and 11.2% mustard-seed meal were compared; after 8 weeks the birds on mustard seed weighed 80 to 90 g. less than did the controls (55). Other experiments compared mustard-seed meal at 0, 3, 6, and 9% as replacements for meat meal in chick starter rations containing 18% protein; growth was normal at all levels (56).

c. Sheep

The only available information on sheep states that mustard-seed meal compared favorably with linseed meal in the rations of ewes, except for palatability (57).

III. POPPY-SEED MEAL

1. General Information

Poppy seed is cultivated in India and Asia mainly for opium production and in Europe and Asia Minor for oil production. The seed contains about 40% of oil and does not contain opium. Caution is necessary in feeding the meal to animals, and it should not be fed to young or breeding animals.

Poppy seed is grown as an oil crop in India, China, Europe, Turkey, Persia, and the U.S.S.R. Before World War II the annual production of poppy seed in Europe, Turkey, Persia, and the U.S.S.R. was estimated at about 50,000 long tons. In 1948 production was about 70,000 tons, and probably somewhat less in 1949; 1954 world production was only about 25,000 tons. The trade in poppy seed is small, and annual exports from the more important sources seldom exceed 10,000 tons. The United States and Germany are the chief importers of poppy seed (1).

2. Botanical Information

The poppy is the only species of the Papaveraceae family which yields a commercial oilseed. The most important species of poppy for oilseed production is the opium poppy (*Papaver somniferum*). One variety which produces white seeds is cultivated in India and Asia mainly for opium production, with the seed as a by-product. In Europe, Turkey, Persia, and the U.S.S.R. a black- or gray-seeded variety is grown for oil.

The small kidney-shaped seeds are contained in a capsule, which is the source of opium; the seeds contain no opium. The seed has an oil-rich endosperm containing the embryo, and the oil content ranges from 44 to 50%. Poppy seed oil is classed as a semi-drying oil (iodine value 136) and is used for edible and industrial purposes. It contains over 60% linoleic acid and about 30% oleic acid.

3. Composition and Processing

There is little information in the literature on the composition of poppy seed. Samples of poppy seed examined by a large United Kingdom processor over the years 1950–56 contained 41.1% oil, 8.9% water, and 3.7% free fatty acid (on oil). A typical poppy-seed cake will contain approximately 37% protein and 10% oil; further details are given in Table II. No information is available on the amino acid composition of poppy-seed protein.

The following information relates to the very small quantity of poppy seed processed in the United Kingdom. The seed is processed in a high-pressure screw press without any pretreatment other than cooking to between 150° and 200°F. The cake contains about 8% oil and 2% water; solvent extraction can be employed to obtain a further yield of oil. The resulting meal contains about 1% oil.

4. Uses

The extracted meal has a high content of digestible protein, but the starch equivalent is low, owing to the complete indigestibility of the fiber and the low digestibility of the fat (58). The commercial meal may have toxic properties owing to the opium alkaloids which arise from contamination of the seed with particles of the capsule (37).

a. Dairy Cattle

Early work indicated that poppy-seed meal had detrimental effects when fed to dairy cattle. For example (53), the feeding of 1.5 kg. per head per day resulted in soft butter fat and a slight depression in milk

fat content. In order to overcome palatability problems, the meal was mixed with other feeds. Other workers (59), however, found that mixtures of poppy-seed meal with oats gave milk and fat yields similar to those of soybean meal, and there were no toxic effects. In 1950, it was reported (60) that poppy-seed cake could not be used as a concentrate feed, but later it was shown (61) that poppy-seed cake (80%) with dried sugarbeet pulp (20%) was satisfactory for dairy cattle. Some authorities (37) recommend a maximum level of 1 to 1¼ kg. per head per day for dairy cows but not for young or breeding animals.

b. Sheep

According to one report (58) sheep tolerated up to 2 kg. per head per day, and the digestibility coefficient of the crude protein was 80%.

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CHAPTER 21

LINSEED OIL MEAL

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I. INTRODUCTION

Linseed oil and linseed oil meal are produced by crushing the seeds of flax (*Linum usitatissimum*) under high pressures, with or without heat, to squeeze out the oil; or the partly crushed seed is extracted with solvents and the oil recovered by distillation. One bushel (56 pounds) of flaxseed contains about 20 to 22 pounds of linseed oil, the residue being linseed oil meal. The oil is the most valuable part, normally constituting about 70% of the value of the seed. [See Table I (1-2).]

Flax, or linseed is one of the oldest crops known to man, and the botanical name, meaning "most useful," is a good one. Numerous references to flax and linen are to be found in the Bible and other ancient writings. Remains of flax plants have been found in Stone Age dwellings, and linen was a principal material used for clothing by the Egyptians and Babylonians four thousand years ago (3).

The origin of *Linum usitatissimum* is not known. Many students believe it originated from *Linum angustifolium*, a wild flax found in the Mediterranean area. Others believe it to be a natural hybrid of other wild flaxes (4, 5). The varieties used by plant breeders today come from India, Russia, and the Mediterranean area. These may have been spread by ancient peoples and adapted to local conditions, but man's association with the plant is so old we can only guess. The older varieties grown in the American Great Plains are mostly of Russian and Belgian origin, whereas the varieties grown in California came from India. Argentine varieties came from various places in Europe.

Flax was used mainly for food and fiber in its earliest days, though the ancient Egyptians used some linseed oil as a preservative and sealing material. The earliest documented use of linseed oil in paint and varnish occurred in the fifteenth century when a monk named de Tholeto formulated a modern-type varnish and the Flemish Van Eyck brothers used linseed oil in portrait painting. Even then, linseed oil was a by-product of linen manufacture,

at least in Europe and Africa. Cotton was expensive, and linen and wool supplied most people with clothing. The use of linseed oil in protective and decorative paints became important in the eighteenth century, and the invention of the cotton gin in 1792 reduced the price of cotton so that the demand for linen fell off rapidly. This caused a shift from fiber production to oil production, and varieties with good yields of seed became more important.

Flax seeds were brought to the United States by the earliest settlers, with orders from their sponsors in Europe to establish a flax industry. In 1658 the Virginia Assembly offered a subsidy for growing flax, but the acreage did not become large until the middle of the eighteenth century.

TABLE I
RELATIVE COMMERCIAL VALUE OF OIL AND MEAL FROM 1 TON (2000 LB.) OF FLAXSEED

Year	United States (Minneapolis) ^a				Argentina ^b		India ^c	
	Oil		Meal		Oil	Meal	Oil	Meal
	Dollars	%	Dollars	%				
					(%)	(%)	(%)	(%)
1930	51.50	72	19.60	28				
1940	48.20	76	14.85	24				
1945	80.00	75	26.00	25				
1950	116.80	77	34.20	23	81	19		
1955	79.80	72	30.80	28	80	20	65	35

^a U. S. Dept. Agr. Yearbook Agr. and Agr. Stat. (1930-1955).
^b Data from Rodolfo C. Antonissen, Buenos Aires. The fact that all Argentine meal is exported may account for its relatively low value.
^c According to Indian Central Oilseeds Comm. 8th Ann. Rept. Year Ending March 1955, Hyderabad (DN), September, 1955. As the crushing industry becomes mechanized and a higher percentage of oil is removed, the relative value of linseed oil should increase in India.

In 1901, H. L. Bolley of the North Dakota Agricultural College showed that "flax-sick soil" was simply infested with *fusarium* wilt and had not been robbed of fertility as growers formerly believed (6). Until that time, flax had been a migratory crop in the United States, grown on newly broken soil for a year or two until the wilt became serious, and then moved to fresh soil again. The processing industry moved with it; plants which had been operating on the east coast about 1800 gave way to plants in Pittsburgh, Buffalo, Cleveland, and Dayton. The latter city became the center of the industry after the Civil War and remained so for many years. Continued westward movement carried the industry to Chicago, Minneapolis, and Fargo. A few plants appeared in Kansas and Nebraska.

In 1903, Bolley introduced wilt-resistant flax varieties and is credited with having saved the American flax industry thereby. From

that time on, the production of flax was stabilized in growing areas such as the American Great Plains, Argentina, and suitable areas of India, Russia, and elsewhere. Minneapolis became the center of flax processing in the United States, a position it still holds. Eastern seaboard plants continued for a long time to import flaxseed from Argentine and elsewhere and to export the cake and meal to Europe; but since World War II these plants have turned to other materials and the importation of flax into the United States has nearly ceased except from Canada and Mexico. California-grown flax is processed locally, and a small amount is raised and processed in southern Texas. The California and Texas plants operate on other oilseeds in season.

Flax was introduced into Argentina from Spain about 1800, and later introductions were made from Italy, France, and Russia. Growth of the industry was slow until about 1880, but after that it became increasingly important until the 1930's, when Argentina produced a large share of the world's supply of flaxseed (7). During this time an Argentine flax type known as Malabrigo emerged, from which several varieties have been obtained by breeding and selection. These newer varieties, such as Querandi M.A., Buck 3, Buck 114, and Klein 11, are replacing the original Malabrigo.

Flax has been grown in India and Russia since ancient times.

II. PRODUCTION AND TRADE

Total world production of flax remained fairly constant from 1935 to 1955, although the distribution varied widely, as shown in Table II (8, 9); production increased sharply in 1956.

In the United States, the principal flax-raising states are North Dakota, with 51% of the total U. S. crop in 1953, and Minnesota with 25%. Following these are South Dakota with 17%, California with 2%, and Iowa with 1% (10). These figures vary from year to year as weather and price conditions change. The average yield in the United States is about 7 bushels per acre (8 bushel per acre in 1953), local yields ranging from 5 bushels per acre in North Dakota to 30 bushels per acre in the Imperial Valley of California. [One large field in California produced over 75 bushels per acre in 1950 (11).]

Varieties grown in the United States include B-5128, Marine, Redwood, and Sheyenne in the Plains States; and Imperial (a selection from Punjab) in California and Arizona. A few older varieties such as Dakota, DeOro, and Victory are also grown extensively but are being supplanted by the higher yielding recommended varieties.

Prices paid in Minneapolis for flax and linseed oil and meal have followed the general price level closely through the years. The price

of flaxseed usually changes slowly, being fairly constant throughout a single crop year; this is also true of linseed oil, which is sold on contracts several weeks or months in advance of delivery. The price of linseed oil meal, on the other hand, fluctuates widely from month to month, being lowest during the period from May to August when cattle are on pasture, and increasing to a peak in December and January. The winter price is commonly 50% higher than the summer

TABLE II
WORLD FLAXSEED PRODUCTION

Country	Short tons		
	Average ^a 1935-39	Average ^a 1945-49	1954 ^{b,d}
United States	308,000	1,095,000	952,000
Canada	42,200	259,000	257,000
Mexico	3,110	36,700	54,000
Argentina	1,670,000	882,000	436,000
Uruguay	109,000	109,200	57,000
India	506,000	429,500	356,000
U.S.S.R.	900,000	414,000	950,000 ^c
Europe (except U.S.S.R.)	148,400	204,600	208,000
Asia (except U.S.S.R., India, and China)	23,500	59,700	29,000
Africa (principally Morocco)	14,000	70,400	81,500
Australia and New Zealand	476	5,600	9,500
Estimated world total	3,740,000	3,600,000	3,450,000

^a U. S. Dept. Agr. Foreign Agr. Service Circ. FFO8-55 (Apr. 25, 1955).

^b Food and Agr. Organization U.N. Monthly Bull. Agr. Econ. & Stat. 4 (10), 39 (1955).

^c Estimate.

^d World production increased in 1956 to 4.8 million tons; production in Argentina (673,000 tons) and Canada (965,000 tons) increased greatly over previous years. See U. S. Dept. Agr. Foreign Agr. Service Circ. FFO5-57 (May 13, 1957); *ibid.*, FFO13-57 (1957).

price. The average farm price of flaxseed was \$1.35 per bushel from 1931 to 1935, and \$4.97 per bushel from 1946 to 1950, reaching a peak of \$6.15 in 1947 under Government price regulation. Production of flaxseed reached a peak of 1,528,000 short tons in 1948. Meal prices ranged from a low of \$19.15 per ton (bagged, FOB Minneapolis) in December of 1934 to a peak of \$89.25 per ton in January of 1947. The highest volume of United States meal production was 997,000 tons in 1943 (10).

In Canada, most of the 1953 crop was grown in Manitoba (106,400 tons), Saskatchewan (98,000 tons), and Alberta (56,000 tons). Average

yields were 10.5 bushels per acre. The average price in 1952-53 was \$3.16 bushel, but it has remained fairly steady at about \$2.50 from 1953 to 1955. Canada usually crushes about 112,000 tons; in 1948, a peak of 177,000 tons were crushed (8). Varieties commonly grown in Canada are Rocket and Marine.

India produced 402,000 tons of flaxseed in the 1952-53 season, of which 148,000 tons was grown in Uttar Pradesh. The average yield was 6.7 bushels per acre in Uttar Pradesh and 4.2 bushels per acre in India as a whole (8). The price in Bombay was equivalent to \$3.13 per bushel on January 23, 1954, and \$2.25 per bushel on January 22, 1955. Varieties commonly grown in India are Small, Bombay Bold, and Calcutta Bold, with some selections from Indian experiment stations, including Albidum, Sativum 121, and Commune 55.*

In Argentina, production is located mainly in the provinces of Entre Rios, Buenos Aires, Santa Fe, and Cordoba, the acreage distribution varying with weather and price conditions. The average yield in Argentina was 10 bushels per acre in 1953 and 1954; a record crop of 2,490,000 tons was grown in 1931-32. Most of Argentina's 1954 flax crop was crushed domestically, with only 4850 tons being exported, in contrast with a 1,650,000-ton annual export before World War II. The crushing industry is located mainly in Buenos Aires and Rosario. Most of the crop is crushed by hydraulic and mechanical screw presses, but solvent-extraction plants are replacing some of these. Nearly all the linseed oil meal and cake are exported to Europe (8).†

Recent data are not available for Russia, the other large flaxseed producer.

III. THE FLAX PLANT AND ITS GROWTH

1. General Structure and Behavior

Flax is an annual, fibrous, woody-stemmed, dicotyledonous plant, upright growing (except for certain winter varieties), with small, straplike, sessile leaves (see Fig. 1). Seed varieties are bred for maximum branching and are comparatively short. Fiber varieties grow tall, with few branches. [Cultural methods have a decisive effect on branching habit, thick stands showing much less branching than thin stands (4).] Most seed varieties are blue-flowering, though some (particularly the yellow-seeded) have white, pink, or lilac blossoms; and a flax field in full bloom rivals anything else in nature for beauty. The flowers are self-fertile, and little natural cross-fertilization takes place.

* Paul E. Quintus, Foreign Agricultural Service, U. S. Dept. Agr., personal communication, 1955.

† Rodolfo C. Antonissen, Buenos Aires, personal communication.

The seed is borne on the tips of the branches in round "bolls," each containing from one to a maximum of ten seeds. (Ten is the usual number.) Except in ideal growing seasons, many varieties continue to bloom and set seed, so it is not unusual for a single plant to bear fresh blossoms and ripe seeds at the same time, with all stages of maturity in



FIG. 1. Flax plant.

between. Fortunately, the ripe seed does not shatter readily, and harvest may be delayed safely until the later seeds ripen. This fact is utilized in California's Imperial Valley, where the Punjab and Imperial varieties are grown with irrigation. After a seed crop is set and ripened, a liberal application of water and nitrogen fertilizer causes a second crop to set. This is allowed to ripen, and both crops are harvested as one. Short-season flaxes, including all Great Plains and Argentine varieties, are selected for a more determinate flowering habit; and the

after-blooming is less pronounced. Such varieties include Marine, Sheyenne, DeOro, and Malabrigo.

Flax straw consists of a pithy center surrounded by bundles of fine longitudinal fibers, which are in turn covered by a layer of bark. The long fibers in this ring are used in making linen from the fiber varieties. The straw from seed varieties is used to a limited extent in the manufacture of cigarette and bank note paper.

2. Gross and Micro Structure of Flaxseed

The flaxseed is flat, oval, and pointed on the end attached to the ovary (see Fig. 2). Seeds of commercial varieties range in length from 4 to 7 mm., the average being about 5 mm. long, 2.5 mm. wide, and

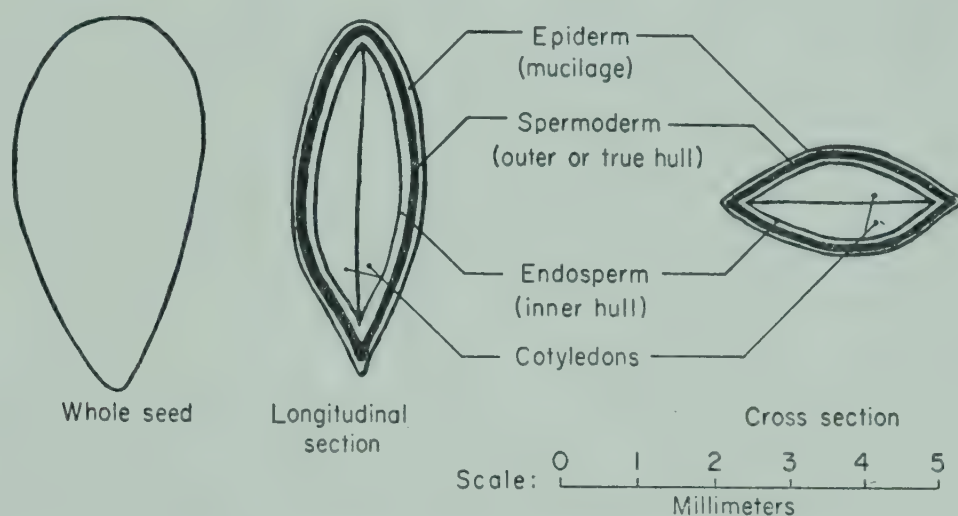


FIG. 2. Diagram of the structure of a flaxseed.

1.5 mm. thick. Weights range from 3.5 to 11 mg. per seed, with an average of 4.5 to 7.5 mg. Density of the seed is 1.10 to 1.15 g./cc.

The outer coating of the seed, or epiderm, is a layer of mucilage, about 0.1 to 0.2 mm. thick. This is a complex mixture of carbohydrate materials which disperses readily in water to form a thick slime. The layer is formed from a starch-like material which is deposited on the seed in a membranous coating. As the seed matures, the material hardens and loses its starchy character (12, 13). Next beneath the mucilaginous epiderm is the spermoderm, or true hull, which is made up of four layers of cells totaling about 0.2 mm. in thickness. First is a layer of round cells, next a layer of longitudinal fiber cells, then a layer of transverse fiber cells, and finally a layer of square, hard, dark-colored pigment cells. This four-layered hull is tough and fibrous, and contains little or no oil or protein. The next layer is the endosperm, or inner hull, which is 0.2 to 0.4 mm. thick and contains some oil and protein. It is difficult to separate it from the true hull, and the two are usually considered together for the type of analysis given in Table III.

Most of the oil and protein are found in the two cotyledons, or seed leaves, which constitute a little more than half the weight of a plump seed. The oil is contained in the cells as microscopic droplets, and these cells must be ruptured in processing in order to secure complete extraction. Most of the protein is contained in separate "aleurone cells" which serve as reservoirs of nitrogen for the new seedling. The rest of the protein is in the building material of the plant cells themselves.

TABLE III
TYPICAL GROSS CHEMICAL ANALYSIS^a OF SEED, HULL, AND MEATS

Constituent	Weight of fraction			Analysis	
	In seed (g.)	In hull (g.)	In meat (g.)	Of hull (%)	Of meat (%)
Protein ($N \times 6.25$)	24	8	16	20	28
Oil	38	3	35	7	58
Fiber	6	5	1	12	1.5
Moisture	10	6	4	15	7
Other	22	18	4	46	5.5
	100	40	60	100	100

^a The separation of hull from meat is difficult and uncertain, variation from one seed to the next is great, and different analysts disagree on interpretation of the results. The values given above should not be applied rigidly.

3. Response to Environment

Most of the seed selection and hybridization being done on flax is for the purpose of increasing yield by developing resistance to disease, parasites, and adverse climate. Work on amount and quality of oil and protein has lagged, because flaxseed is bought and sold on a grading system which provides no premium for oil quality or quantity. However, some parallel work is now being done to improve quality and quantity of the oil and protein in the seed.

New techniques of tillage, crop rotation, and weed control have had an effect on yield and quality. In general, flaxseed grown in the Great Plains of the United States from 1944 to 1954 had a higher percentage of oil and a lower percentage of protein than flaxseed grown during the preceding decade.

The effect of climate and weather on flaxseed quality is complex and related to variety. Seeds well adapted to the climate of one country may not do well in another, and the effect of climate on yield in the two countries may be quite different. For example, Punjab (selected from an Indian flax) is well adapted to California conditions with irrigation and produces seed which is superior for the production of oil of high iodine number but some

what inferior to northern United States varieties for quantity of protein. On the other hand, Punjab does not grow well in Texas or the Great Plains.

For any given variety, high temperatures and low rainfall during the growing season cause lower oil content, oil of lower quality, and higher percentage of protein in the oil-free meal (14-16). Of particular importance is the temperature during and immediately after the onset of blooming. Excessively hot weather at this time causes shriveling and lowering of both bushel yield and oil percentage. The effect on protein quality, if any, is not known.

Time of planting naturally varies greatly, depending on where the flax is grown and where it appears in the crop rotation scheme. Apparently, planting date has little or no effect on seed quality as long as the seed matures before frost and avoids extremely hot weather during its formation (17-19). It does have a very marked effect on yield, early planting giving much better yields in some areas.

Weather conditions during harvest may have a marked effect on seed quality after the seeds have formed and ripened (19). Warm, dry weather permits rapid harvesting and promotes maximum quality, though too-dry conditions cause the seed to become brittle so that some mechanical cracking occurs. Damp weather or prolonged delays during harvest, with consequent fungus growth, heating, or sprouting, cause loss of protein and deterioration of oil quality. In addition, sand and dust adhering to the damp seed increase the ash content of the meal and cause undue wear on processing machinery.

Seed changes occurring during ripening continue from blossoming to harvest and perhaps longer (17, 18, 20, 21). In the milk stage, most of the nitrogen is already present but in the form of amides rather than proteins. Very little fat is present. As the seed yellows, the amides change gradually to proteins and the oil deposits are formed rapidly. At full ripeness, about 60% of the nitrogen has been converted to proteins, with the remainder present as glutamic and aspartic acids and other nitrogen derivatives, and the fat has increased to its final value, becoming more unsaturated. The sugar content decreases during this period, being replaced by fat in the endosperm and cotyledons, and by mucilage on the seed coat. The ripe seed contains no starches or reducing sugars. There is evidence that oil content and iodine number increase after harvesting and during storage (21).

IV. PROCESSING

1. Harvesting, Cleaning, and Storage

Flax is harvested and threshed by conventional methods employed with cereal grains, the choice of method depending on local conditions or customs. In North and South America, most flax is harvested by first cutting and windrowing, and then threshing with a pickup combine. In India, most flax is cut by hand with a sickle and threshed by dragging weighted mats of tree branches over the heads on a threshing floor.

Flax grown for fiber is pulled by hand before it is ripe, and subsequent operations are carried out to prevent damage to the stems.

By its nature of growth, flax is subject to intense weed competition; and most flaxseed as harvested contains from 5 to 50% weed seeds which must be removed before crushing. Most of these are readily removed. The hard-to-remove weed seeds are usually high in fiber content and low in protein. By far the worst of these in the Great Plains area of the United States is yellow foxtail (*Setaria lutescens*), otherwise known as pigeongrass or watergrass. It is almost impossible to remove this seed completely without severe losses of cracked and lightweight flaxseed.

Flaxseed is safely stored when its moisture content is below 10.5% provided the relative humidity of the surrounding air does not exceed 75% for long periods (22). The usual practice in handling flaxseed of high moisture content is to crush it immediately or else dry it by "turning" from one bin to another. In some instances hot-air dryers are used.

2. Oil Extraction

a. Mechanical Processes

The earliest known method of linseed oil extraction was by boiling the ground seed in water and skimming the oil off the top. Later, crushed and steamed seed was placed in cloth bags and squeezed in a hand-operated lever or screw press. This method still persists with modifications in the hydraulic process; but the hydraulic press is no longer used on flaxseed in America and is being replaced elsewhere by more efficient processes where power is available. This process left 5 to 10% oil in the press cake.

The mechanical screw press, invented in 1904, succeeded the hydraulic press in crushing flax, with consequent saving in manpower and with improved oil removal (4 to 7% residual oil). Linseed oil meal made in the mechanical screw press is dark brown in color, partly from the heat introduced in the tempering cookers and in the presses, and partly from the oil left in the meal. It has a "toasted" odor and is quite palatable. Since the moist, plastic, ground seed is molded into cake at high temperature and pressure and then ground, any hulls, weed seeds, and foreign materials present are firmly embedded in the mass and are broken up in grinding. Thus, screw-pressed meal is notable for its homogeneous appearance.

In India, a large amount of flax is crushed in the *Kohlu* (see Fig. 3). This consists of a conical chamber from 1 to 3 feet in diameter, usually of wood but sometimes of iron or steel, inside of which is fitted

another solid cone of wood or steel so that there is a narrow annular space between them. Sometimes a hollowed-out tree stump serves as the outer chamber. The inner cone is rotated, usually by a bullock at the end of a sweep arm, while flaxseed is fed into the annular space. Oil is

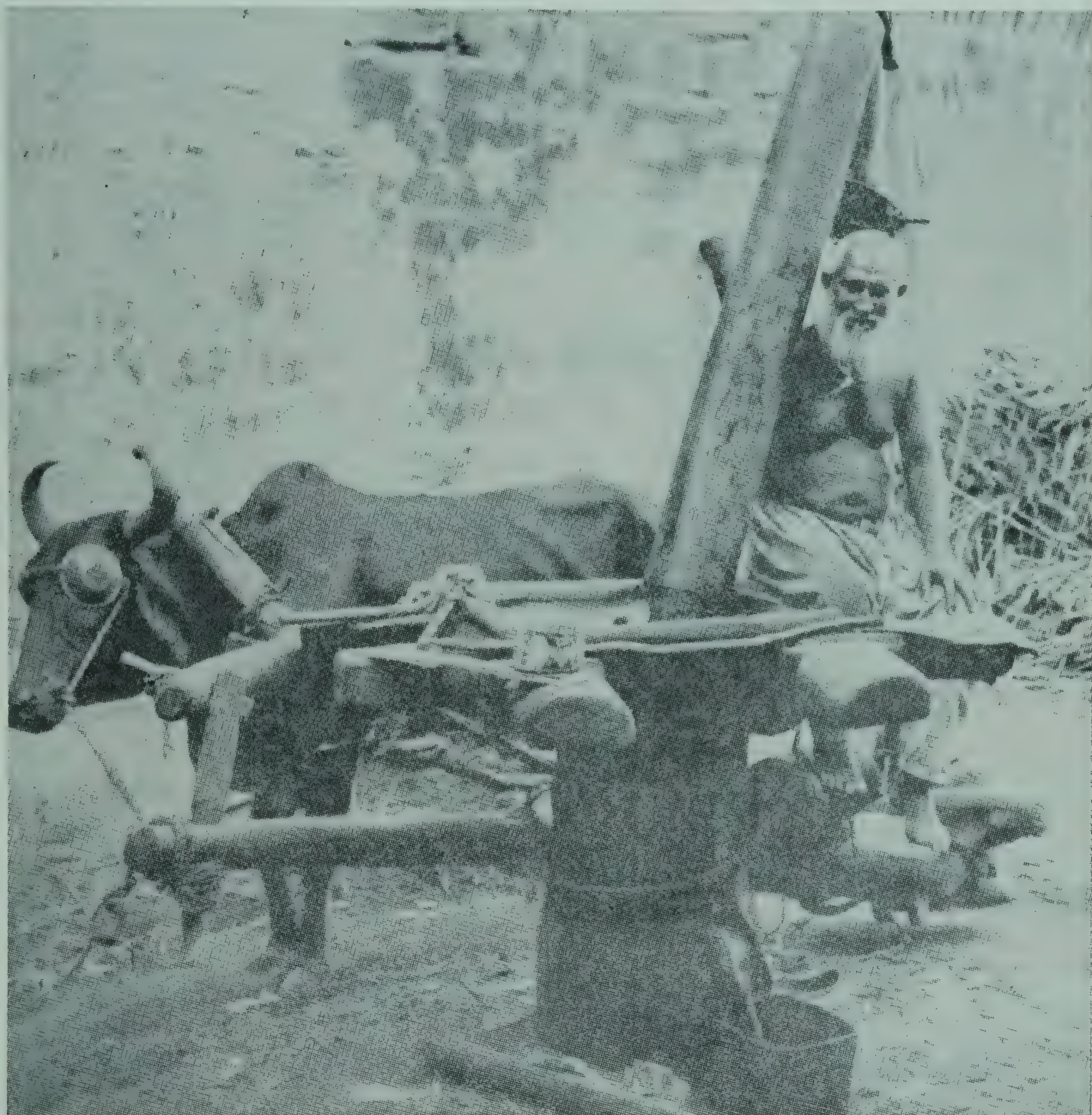


FIG. 3. A typical village *Ghani* or *Kohlu* used for removing oil from oilseeds. (Photograph courtesy of Ministry of Food and Agriculture, Government of India.)

squeezed from the seed and runs out at the bottom, leaving a cake with about 20% oil content. Larger metal installations are rotated with electricity instead of bullocks.

b. Solvent Extraction

Attempts were made in Europe before 1900 to extract fats and oils by means of petroleum naphthas. These early extractors were of the batch type and were not very successful, partly because of inefficient

oil removal but principally because the solvent odors could not be eliminated completely and the resulting meal was not palatable to livestock. Most of these difficulties have since been overcome, and some installations, notably in England and continental Europe, still operate on the batch principle. The batch extraction method was tried on an experimental basis in the United States after 1900, but it produced an unsatisfactory grade of meal and was abandoned without any of the so-called "new process" linseed oil meal ever having been marketed commercially.

The first successful continuous-process solvent extraction of flaxseed was begun in Minneapolis in early 1949, with hexane as a solvent. It

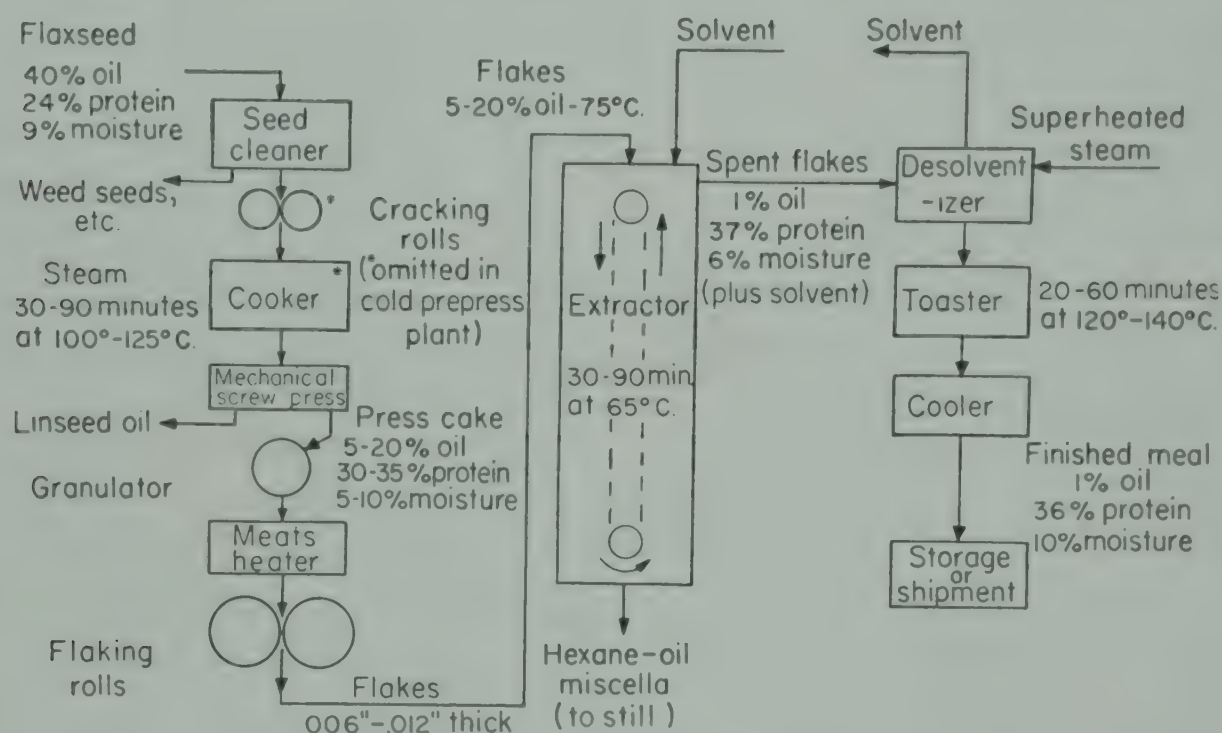


FIG. 4. Flow sheet of prepress solvent extraction of linseed.

proved so successful that by 1955 most of the mechanical pressing plants operating on flaxseed were replaced by solvent plants. Since flaxseed is high in oil content, the flakes formed from whole seeds are too fragile to extract directly, such an excess of fine material being formed as to plug the equipment. This is overcome by "prepressing" the flax in mechanical screw presses to remove a little more than half the oil and reduce the residual oil content to 10 to 20%. This cake is then tempered with steam, flaked, and extracted in a variety of equipment, all designed to move the flakes against a continuous counterflow of solvent. Residual oil content is thus reduced below 1%. Some plants grind and heat the seed before prepressing; others omit these steps (23). A flowsheet of the process is shown in Fig. 4. The intervals and temperatures of cooking which are shown are typical but vary a great deal from one plant to another. (See Chapter 4 for a general discussion of oilseed processing.)

Processors, equipment manufacturers, and government laboratories continue to conduct experiments on total extraction of linseed without prepressing, and at least one plant has been built and is operating on this basis; but most of the extracted linseed oil meal is produced in plants using prepressing. In 1951-52, a little more than half the total processing in the United States was done in screw presses, with about 3% processed by extraction without prepressing. By 1955, the portion processed by all types of solvent extraction had increased to 80%.

3. Meal Treatment

a. Toasting

In the solvent-extraction process, the flaxseed is never subjected to the temperatures and pressures involved in the mechanical screw process. Consequently, the meal has a considerably different appearance, characterized by lighter color and a separation of constituents. In order to darken the color, agglomerate some of the dusty fines, and remove excess moisture, the meal is heated in a "toaster" after the extraction step. Even so, the meal remains lighter in color; and any weed seed parts, flax hulls, or foreign material which may be present show up much more prominently than they would in the darker, harder screw-pressed meal, even though present in the same amount. Such separation does not affect the feeding value of the meal. The effect of the heating on feeding value is disputed, with little evidence for effects in either direction (24, 25). This is in marked contrast to soybean oil meal, whose value for feeding some animals is greatly improved by heating.

b. Sizing, Pelletizing, and Crumblizing

Linseed oil meal cakes made by the hydraulic process were hard and, when broken up and ground in a coarse attrition mill, gave rise to material with a wide range of particle sizes. This mixture was usually screened, the fine material passing through about a 14-mesh screen being sold as fine meal and the coarser material being sold separately as "pea-size." The pea-size meal served a useful function in feeding range cattle and sheep with a minimum of waste, but it has practically disappeared in the United States along with the hydraulic process.

As a replacement, and to fill a greatly increased demand, large amounts of meal are made into pellets by steaming and forcing the meal through metal dies under heavy pressure, a process which is standard throughout the feed industry. Some of the pellets may be

coarsely cracked between rolls to produce crumbles, a much coarser and more homogeneous product than the original fine meal. A part of the increasing demand for pellets and crumbles may be due to the increased amount of dusty fines appearing in solvent-extracted meal. Elimination of these fines reduces the nuisance and loss connected with the handling of dusty meal, and makes the product more palatable to livestock.

V. COMPOSITION

1. Appearance and Physical Character

Linseed oil meal is distinguishable from other oilseed meals by the presence of pieces of flaxseed hulls, which are not readily broken up in processing. As was pointed out previously, these are not so apparent in screw-pressed meal but are prominent in most solvent-extracted linseed oil meals, and larger pieces are readily isolated with a 40-mesh sieve. Anyone not familiar with solvent-extracted linseed oil meal is likely to regard these hulls as foreign material, whereas they are actually a valuable part of the flaxseed, since they contain the mucilage. Also characteristic of small-seeded meals such as linseed is the presence of a few small weed seeds not removable in commercial cleaning. These seeds are always rendered non-viable in processing, either by heat or by solvents, and are usually broken up in grinding the meal. Thus they cannot germinate on the farms where the meal is fed.

Linseed oil meal has a range of particle sizes from less than 15 mesh to more than 400 mesh, the distribution varying with raw material and processing conditions. Typical (though not necessarily average) size analyses of screw-pressed and solvent-extracted meals are shown in Table IV. Composition of the different fractions of solvent-extracted meal varies with the particle size, the finer material in general having the higher protein content. This variation of protein content with size is due primarily to the fact that most of the hulls and impurities are in the coarser fractions. The extremely fine fraction shows a reduction of protein content from the maximum, possibly from inclusion of fine soil dust adhering to the seed. Variation of protein content with particle size is much less apparent in screw-pressed meal, although the size distribution is about the same.

2. Chemical Analysis

a. Gross Analysis

Typical gross analyses of linseed oil meals are shown in Table V (26).

TABLE IV
TYPICAL PARTICLE SIZE AND PROTEIN DISTRIBUTION

Screen mesh size (Tyler standard)	Solvent-extracted linseed oil meal		Mechanical screw-pressed linseed oil meal	
	Weight (g.)	Protein content (%)	Weight (g.)	Protein content (%)
Larger than 10	0.1	22	0.0	—
10-14	0.3	24	2.5	36.6
14-16	3.3	28.5	5.9	36.4
16-18	6.8	27.1	6.5	36.4
18-20	17.8	33.0	6.2	36.3
20-30	27.0	36.2	20.6	36.3
30-40	20.6	38.7	25.8	36.3
40-50	7.9	44.8	9.2	36.3
50-60	5.5	47.4	7.6	36.3
60-80	4.0	50.4	6.0	36.6
80-100	2.2	51.4	4.1	36.8
100-200	2.6	50.2	4.4	38.0
Smaller than 200	1.9	46.4	1.2	39.1
Total	100.0		100.0	
Weighted average		38.1		36.4

TABLE V
TYPICAL GROSS CHEMICAL ANALYSIS OF LINSEED OIL MEALS AND CAKES

	United States ^a		Argentina ^b		India ^{c,d}
	Solvent- extracted (%)	Screw- pressed (%)	Solvent- extracted (%)	Screw- pressed (%)	
Crude protein (N × 6.25)	36.0 ^e	34.0 ^e	33-34	32	31
Crude fat	0.5 ^e	3.5 ^e	1-1.7	5.5-6.5	9.8
Crude fiber	9.5 ^f	10.0 ^f	—	—	—
Nitrogen-free extract	33.0 ^e	32.0 ^e	—	—	36.8
Ash	6.5 ^f	—	—	—	7.2
Moisture	—	—	7-8.5	5-6.5	8.3

^a Guaranteed values shown on labels.

^b Information from Rodolfo C. Antonissen, Buenos Aires.

^c According to A. Khan, *Oil & Oilseed J. (India)* 4 (4), 17 (1951).

^d Other values given by Khan are: fiber, 6.3%; sand, 1.9%; P₂O₅, 1.7%; soda, 0.7%; potash, 1.4%; calcium, 0.21%.

^e Minimum.

^f Maximum.

b. Amino Acids

The amino acid composition of linseed oil meal is shown in Table VI (27, 28).

c. Mucilage

Flaxseed is unique among common grains in being coated with large amounts of mucilage (2 to 7% by weight of dry seed, or 3 to

TABLE VI
ESSENTIAL AMINO ACID COMPOSITION OF LINSEED OIL MEAL

Amino acid	Content of amino acid				(Calculated as g. per 16 g. of nitrogen) ^b
	(%) ^a	(%) ^b	(%) ^b		
Arginine	2.1-2.8	3.2	2.8	8.6	8.6
Histidine	0.5-0.6	0.7	0.6	1.9	2.0
Isoleucine	1.1-1.4	2.2	1.6	5.9	4.9
Leucine	1.8-2.5	2.2	1.9	5.8	5.9
Lysine	0.8-1.1	1.5	1.2	4.1	3.7
Methionine	0.3-1.1	0.4	0.4	1.0	1.0
Phenylalanine	1.7-1.9	1.5	1.4	4.1	4.3
Threonine	1.0-1.7	1.3	1.1	3.6	3.4
Tryptophan	0.5-1.0	0.6	0.4	1.5	1.2
Tyrosine	1.7	0.8	1.4	2.2	4.1
Valine	1.7-1.9	1.8	1.5	4.9	4.7
Nitrogen content	—	5.95	5.24		
Crude protein (N × 6.25)	—	37.2	32.7		

^a According to R. J. Block and D. Bolling, "The Amino Acid Composition of Proteins and Foods," 2nd ed. Charles C Thomas, Springfield, Ill., 1947. Percentages are based on commercial linseed oil meal of 34% crude protein. The range given may represent uncertainties in analysis, or a range in values with different meals, or both.

^b According to H. H. Williams, *Cornell Univ. Agr. Expt. Sta. Mem.* **337** (1955).

10% by weight of the meal). This mucilage, a water-dispersible carbohydrate, consists principally of non-reducing sugars and aldobionic acids insoluble in alcohol or other organic solvents (29, 30). It is nearly indigestible by non-ruminant animals and poultry but is digested to some extent by yeasts and bacteria in ruminants and is considered to be responsible for the particular value of linseed oil meal in feeding cattle and sheep.

Mucilage is easily prepared in the laboratory by soaking flaxseed or linseed oil meal in water, straining off the solid material, and treating the filtrate with a large excess of ethyl alcohol (29). The mucilage

appears as a white, fibrous mass which becomes friable when completely dry. It is readily redissolved in water with the formation of a viscous slime. Protein is extracted from the seed along with the mucilage and must be removed by some reagent such as phosphotungstic acid before precipitation of the pure mucilage with alcohol. Uses of the mucilage will be discussed later in the chapter.

d. Carbohydrates

The carbohydrates present, in addition to the mucilage, are mostly sugars and cellulose. Reducing sugars and starches do not occur in the mature seed (17).

e. Fat

Screw-pressed meals contain 4 to 7% linseed oil, whereas solvent-extracted meal contains 1% or less. This oil is highly unsaturated (iodine number, 160 to 190), has a low melting point (remaining liquid well below 0°F.), and is readily digestible. It has a definite value in feeding because of its high energy content, replacing about 2.25 times its weight of corn. Numerous studies have been made on the specific effect of fat in beef and dairy rations with inconclusive results (31–37).

f. Toxic Constituents

Immature flaxseed contains a small amount of the cyanogenetic glucoside *linamarin* and an associated enzyme *linase*. At certain temperatures (optimum 40° to 50°), conditions of acidity (from pH 2 to pH 8, optimum pH 5), and in the presence of moisture, linase acts on linamarin to release hydrocyanic acid (HCN). When the oil is removed under low-temperature processing conditions, a part of the linamarin and linase remain unchanged in the meal; and they have caused the death of some animals in those areas where linseed oil meal is fed as a slime or gruel. Under normal conditions of manufacture, involving high-temperature treatment at some stage of the process, the linase (and to a large extent the linamarin) is destroyed.

The amount of linamarin varies with variety, maturity, and oil content of the flaxseed. Mature seed contains little or none, which may account for the fact that fiber-flax varieties (which are usually harvested before they are totally ripe) appear to contain more than varieties grown for seed; and seed with low oil content contains more than seed with high oil content (38, 39). The amount appears to be unaffected by storage or by the nature of soil or fertilizer used (40). Solvents used in extracting the oil have no effect (41). The amount present in some linseed oil meals was found by early workers to be equivalent to 200 to 700 parts of HCN per million parts of meal, but a more recent

study on samples collected from various countries shows this figure to be 100 to 300 parts per million (42).*

When dry meal containing linase is eaten by non-ruminants, the enzyme is destroyed by acidic digestive juices before it can react with linamarin. In ruminants, the enzymatic reaction takes place, but the HCN is absorbed into the blood stream quite slowly; and it is eliminated by the kidneys, liver, and lungs so rapidly that the concentration never becomes high enough to cause any ill effects. Thus the danger of HCN poisoning from feeding even large amounts of dry linseed oil meal is extremely small or non-existent (43-45).

g. Protective Constituents

Workers at South Dakota State College have found that linseed oil meal in proper amounts has a protective effect against poisoning due to selenium compounds (46). The exact nature of the protection is uncertain, but linseed oil meal appears to render the selenium non-toxic rather than to prevent its absorption.

h. Phytin and Inositol

Linseed oil meal contains recoverable phytin in an amount equal to about one-tenth of the protein (47). Phytin is readily converted into inositol, a material now obtained commercially from corn steep liquor.

i. Other Minor Constituents

Varying small amounts of calcium and phosphorus and some vitamins and vitamin-like materials are contained in the meal. An assay of one sample of solvent-extracted linseed oil meal manufactured from flaxseed grown in the Great Plains of the United States shows the following: calcium, 3.9 mg./lb.; phosphorus, 9 mg./lb.; vitamin A, none; thiamine, 4 mg./lb.; riboflavin, 0.0018 mg./lb.; niacin, 0.046 mg./lb.; pantothenic acid, 0.014 mg./lb.; and choline, 2.2 mg./lb.†

VI. USES OF LINSEED OIL MEAL

1. Livestock Feeding

a. Ruminants

Linseed oil meal has long been regarded as an ideal protein concentrate for cattle and sheep. Its use results in improved health, superior milk production, markedly improved carcass grade and finish, and sleek appearance. These results are well out of proportion to the amount of

* Collection and analyses by J. W. Hayward, Archer-Daniels-Midland Company.

† Assay performed by the Wisconsin Alumni Research Foundation.

protein and total digestible nutrients contained in the linseed oil meal and are characteristic of this meal.¹¹

Surprisingly, this wide acceptance has, until recent years, resulted in a lack of serious investigation into the properties and constituents of linseed oil meal as a cattle food. Other feeds were investigated as substitutes for linseed and thus received much more study in the laboratory and feed lot. Since the advent of the solvent-extraction process in the linseed industry and the emergence of soybean oil meal as a dominant factor in the feed industry, linseed oil meal as a cattle feed has been studied more intensively.

The digestibility of linseed oil meal protein by cattle is not affected by the amount of other feed consumed (48). Thus it is utilized as efficiently by fat or high-producing cattle as by those fed a subsistence ration. The fact that linseed oil meal contains less lysine and methionine than some other meals does not affect its value for cattle, since rumen microorganisms are able to synthesize vitamins and essential amino acids not present in sufficient amounts in the original feed.

Linseed oil meal absorbs large amounts of water (solvent-extracted linseed oil meal absorbs eight times its own volume) and increases in bulk proportionately. When this bulking takes place in the animal's rumen, feed is retained there longer, thereby giving the rumen bacteria and yeasts more time to soften and digest the nutrients. No other feed has this water-absorbing quality to the same degree as does linseed oil meal. The bulkiness of the linseed oil meal ration, together with the lubricating quality of the linseed mucilage, gives the ration a pronounced beneficial effect on the intestinal tract by protecting the walls from harsh fibrous materials and facilitating the continuous movement of the mixture through the intestine. It thus serves to regulate excretion, preventing constipation without undue "looseness." Contrary to popular opinion, linseed oil meal is not laxative in the sense of being cathartic, and large amounts may be fed without ill effect (49, 50).

The author personally fed a herd of twelve Holstein dairy cows an entire Minnesota winter on solvent-extracted linseed oil meal, alfalfa hay, and mineralized salt, with some of the cows receiving as much as 20 pounds of linseed oil meal per day, each. Milk production was high and their health remained good, although they lost a little weight between December and May. None of the cows showed any digestive disturbance or loss of appetite. This result bears out the experience of others at Michigan State College, Cornell, and elsewhere (50).

A typical dairy ration, as recommended by the University of Minnesota, is presented merely as an example. This ration is to be fed with medium-quality mixed hay, with or without silage, and with salt and minerals as needed:

Ground corn and cob meal	200 pounds
Ground whole oats	200 pounds
Wheat bran	100 pounds
Linseed oil meal	100 pounds

Commercial feed mixers supply dairy rations containing linseed oil meal plus materials such as minerals, molasses, and urea; and these mixtures may be cheaper and more efficient when properly used.

Sheep and goats are efficient users of roughages; protein concentrates are not usually fed them in large amounts when good legume hay is available. When poorer roughages are used, or when it is desired to fatten lambs for market, linseed oil meal is an efficient source of easily digested protein; and the conditioning, healthful effect of linseed oil meal improves carcass grade and keeps the animals from going "off feed" (51).

A number of investigations have been made into the comparative feeding value of solvent-extracted and screw-pressed linseed oil meals. Studies at the universities of Iowa, Nebraska, Ohio, and elsewhere indicate no significant difference in feeding value between the two meals either as to digestibility or efficiency (52-55). The presence of the extra fat in screw-pressed meal, however, was sufficient in 1955 to bring a premium of \$4.00 per ton in the United States in spite of its lower protein content. Both types of meal are equally effective in promoting "finish" (52, 54, 56).

b. Swine

Linseed oil meal, being deficient in some essential amino acids, principally lysine and methionine, is not suitable as the sole source of proteins for swine (57-59). A mixture of linseed oil meal, alfalfa meal, and tankage (the famous Wisconsin Trio Mixture), however, makes a satisfactory protein supplement. (This mixture is improved by replacing part or most of the tankage with soybean oil meal.) The linseed oil meal in these mixtures is particularly valuable for swine fattened on rye, since the proteins from flaxseed and rye supplement each other (60).

A typical swine ration as recommended by the University of Minnesota is presented as an example. Here, again, commercial swine feeds containing these ingredients plus minor additives may be more efficient. Rye may be substituted for half the corn in the ration:

Round shelled corn	800 pounds
Tankage	30 pounds
Linseed oil meal	30 pounds
Soybean oil meal	30 pounds
Alfalfa meal	30 pounds
Minerals	10 pounds

c. Poultry

Linseed oil meal is not suitable as a sole source of protein for modern poultry rations but is satisfactory in amounts up to 3% of the total ration. When fed in larger amounts, linseed oil meal has a depressing effect on the growth of chicks and poults. Some investigators have found the growth depression to be caused by the mucilage, which causes the meal to adhere to the chick's beak in a gummy mass (61, 62). This causes a necrosis and malformation of the beak, reducing the chick's ability to eat. Pelleting or coarse granulation of the ration reduces or eliminates the beak deformation. Large amounts of linseed oil meal in chick rations also cause sticky droppings with attendant problems of pasted vents, crusted litter, and balled-up toes.

Other workers attribute the growth depression to an increase in requirements of pyridoxine (vitamin B₆) in the chick ration and have been able to improve the ration either by supplementing with pyridoxine, by soaking and drying the linseed oil meal, or by autoclaving (63-65).

d. Other Feed Uses

Linseed oil meal is used in amounts from 3 to 5% in dog foods and in mink and fox foods to improve elimination and general health of the animals and thereby increase the gloss and quality of the fur. It cannot be used safely in fish foods without being autoclaved or else boiled and dried to destroy the cyanogenetic materials (66). Ground whole flaxseed and linseed oil meal are used in horse rations for general health improvement and to impart a finish to the hair.

2. Human Food and Medicine

Linseed oil meal has not found wide favor as a source of protein for human food, although several tons are utilized each year in specialty breads. The poor acceptance is probably due to the difficulty in cleaning the seed completely. If the demand were great enough this could be overcome, and an acceptable human food from this viewpoint could be made by flax processors.

Ground flaxseed has been used since ancient times in making poultices for suppurative infections, and the practice is still approved by the medical profession. Linseed mucilage has been used successfully in the treatment of peptic ulcers and as a mild laxative. Prior to the advent of home-waving preparations, women used the mucilage as a wave-set for their hair. Linseed oil has been found to increase blood cell sedimentation rates (67).

3. Industrial Uses

Methods have been devised for extracting proteins from linseed oil meal with good efficiency (68). This is a potential source of raw material for plastics and fibers as well as glutamate food flavorings. Linseed oil meal has been used as a constituent of phenolic plywood glues, as a corrosion inhibitor, and as an agent to control sedimentation and flocculation in well-drilling muds (69-71). Some of these uses are quite important, but the amount of linseed oil meal going into them is a very small portion of the total manufactured. Intensive work is being done in the laboratories of some flaxseed processors to diversify these uses and free linseed oil meal from its complete reliance on the cattle feeding industry.

VII. TRENDS

1. Movement of Flax Production Centers

Since the growing area for flax and the feeding area for meal are both nearby, Minneapolis has remained the major flaxseed processing center in the United States. The spread of flax culture to California occurred after suitable varieties were developed which would respond to irrigation with high yields. It emerged in 1939 as an important crop in California, and by 1943, with flax prices high, 40% of the Imperial Valley cropland was planted in flax. Since 1950, however, acreage has again declined rapidly, giving way to more profitable crops. With no virgin land left, the flax-growing industry is not likely to move again in the United States. Present and developing knowledge of means for combating diseases and weeds promise a stable future. [Since 1948, rust has become a major menace in the northern Great Plains, along with the ever-present wilt (72).]

Flax remains an important crop in Argentina, though the acreage has declined severely since World War II. Government policies have had a decided effect on the growth or decline of flax growing and processing in Argentina.

Nearly all India's flax is produced on small farms using hand labor and animal power. Increasing industrialization of the country, being encouraged by the Indian Government, is likely to require greater quantities of linseed oil for paint and soap, and linseed oil meal for feed; and the use of power machinery will undoubtedly increase. Efforts to develop a linen industry have not been successful.

2. Research

For centuries, linseed oil has enjoyed the distinction of being an indispensable drying oil. New techniques are changing this situation.

with other oils (notably soybean) taking an increasingly larger share of the paint and resin market. Similarly, the tremendous increase in soybean oil meal production in the United States [from 1300 tons in 1921 to 5,051,000 tons in 1953 (10)] has begun to compete with linseed oil meal in some of its feed markets.

The use of synthetic urea in beef and dairy feeds has posed a serious threat to the use of linseed oil meal in the cattle feed industry, since a pound of urea supplies as much nitrogen in a ration as 7 or 8 pounds of linseed oil meal and is therefore a much cheaper source of this element. Urea does not have the capacity of linseed oil meal to upgrade carcasses, however, and cannot successfully replace it when cattle are fed for a high-quality market. (See also Chapter 12.)

Another factor likely to affect the demand for linseed oil meal has been the breeding of heat-resistant, Brahma-cross cattle which thrive in warmer climates, and the consequent development of a growing beef and dairy industry in southeastern United States. Since this is in a cotton-growing area instead of a flax area, linseed oil meal is likely to lose a part of its cattle feed market.

Continuation of these trends will force the flax industry to accelerate the development of new and higher-value uses for linseed oil and meal in order to compete. Some of this work is already under way, but most linseed oil meal will be used in animal feeds for a long time to come.

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CHAPTER 22

MINOR OILSEED AND TREE NUT MEALS

J. A. KNEELAND

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I. INTRODUCTION

The factors controlling the production of oilseeds necessarily control the by-products derived therefrom. It is of interest to examine them to determine the cause of the small volume which characterizes the meals discussed in this chapter. Some of the oilseeds to be mentioned are just coming into prominence, others are government-supported surpluses diverted to other channels, and a third classification would include those oilseeds whose production is restricted by the scarcity of economic growing areas or by lack of distribution. Even the great soybean and cottonseed were "minor oilseeds" at one time; and those workers who were inspired to develop the information necessary to produce and utilize these sources of protein contributed a great deal to our food sources.

Safflower is one of the protein sources which is gaining recognition and may, if economically warranted, become a major source in geographic areas of optimum climatic conditions for growth. Almonds and walnuts grow in many areas, but in the United States it is only in California that their production is on a sufficient scale for them to be a by-product meal. The price support of these products in the United States frequently results in a larger production than can be absorbed in normal channels of distribution, and the surplus is occasionally diverted from edible trade channels to oilseed crushing, resulting in a by-product meal of abnormal volume and quality. Such surplus by-products are not regularly available, however. The babassu tree flourishes wild in Brazil; the availability of babassu meal is subject to the demand for babassu oil in Brazil, collection of the nuts, and political and exchange factors influencing exportation of the babassu nut. Political factors have reduced the availability of perilla and hempseed meal, for these two oilseeds are found most abundantly in Manchuria.

This chapter will provide the background on a few minor oilseeds,

some of which offer promise of growth in volume. No attempt is made to include all minor oilseeds and tree nut meals of the world.

II. SAFFLOWER MEAL

1. History

Safflower is one of the oldest oilseeds; it was found in the tombs of the Pharaohs (1). Then, however, the safflower plant was grown for its flower which yielded the dye carthamin, a brilliant but fugitive scarlet dye for silk. Because the seed of ancient times was quite low in oil content and high in fiber, it did not flourish as an oilseed. Safflower found its way from Egypt to many countries. The country of the greatest area of planting is India, where over a million acres were planted in 1948 (2).

There were many attempts to develop the crop as a source of oil in the United States during the period 1900-35 (3). From 1935 to 1937 there were 500 to 4000 acres grown in Montana, with discouraging results (4). During the 1940's there were several attempts to grow the crop in the midwestern area on a larger scale. It was in this period that new varieties were developed which are responsible for the crop's larger volume in California in 1956.

Other countries where safflower is grown are China, Japan, Australia, Turkestan, France, Germany, Italy, Russia, Spain, Iran, and the Philippines. Production in each country is primarily for local consumption, and only recently has any substantial volume moved in export trade.

The seed is grown for its oil content, which is 24 to 40%. The oil is semidrying, with an unusually high content of linoleic acid (over 70%) and practically no linolenic acid. In the United States the oil is at a premium because of its light color and non-yellowing character; alkyd resin manufacture consumes the major portion. In countries where the demand for edible oils is greater than for drying oils, safflower oil is used as an edible oil.

2. Production and Trade

Because of its relatively higher oil content, safflower is being grown in increasing quantities (5). New varieties developed in the great northern plains of the United States have not expanded into large tonnage because of lack of organized support of the crop in that area. The California development has grown at a faster rate, partially because of better yields and growing conditions. In 1950, for example, 25,000

acres yielded 4000 tons of seed (520 tons of protein), but in 1956, 84,000 acres yielded 70,000 tons of seed (9,150 tons of protein).

Yields of seed per acre are a function primarily of the amount of water available during the growing season, although variety, weather conditions, and type of soil are also important. Dry-land farming will produce from 250 to 2500 pounds of seed (30 to 325 pounds of protein) per acre. Optimum water conditions can increase this yield to 1000 to 4000 pounds of seed (130 to 520 pounds of protein) per acre. Safflower is an established crop in northern California and has a definite place in crop rotation programs. In 1955, for example, 45% of the crop was planted directly after rice harvest. Economically, it has given good returns, particularly on land of high water table.

The price of seed in the United States has been guaranteed each year by an oilseed crusher and has varied from \$70 to \$100 per ton of seed delivered in the San Francisco Bay area. The price of the whole pressed safflower seed has fluctuated with the seed price as well as with that of other oilseed meals. Its price in 1954 and 1955 was in the range of \$30 to \$35 per ton. This price, \$1.65 to \$1.95 per unit (1% or 20 pounds per ton) of protein, places it among the less expensive protein sources. The price has been relatively steady compared to the normal commodity price fluctuations. Prices on midwestern material have been \$40 to \$50 per ton when available, but the amount of meal produced in the Colorado-Nebraska area was less than 4000 tons in any one year. The Japanese and Indian prices are in the same range as the California prices.

In India, the cake produced by hydraulic pressing or continuous screw-pressing is used for feeding buffaloes, oxen, and cattle. In southern India, it is used as a fertilizer on the coffee plantations. In Japan the meal has been used by the poultry and dairy trade.

3. Seed

The safflower plant, *Carthamus tinctorius*, is a hardy, erect herb, related to the thistle, which grows 18 to 40 inches in height. It has a coarse central stem, branched at the top, each of the many branches terminating in a head. These heads, which contain the flower, usually blossom after a period of 75 days and contain 20 to 100 individual florets. Each of these florets may bear one smooth, white or cream-colored seed which ripens within 100 to 120 days after planting (6). Botanically, the seed of the safflower is known as an achene. The seed will average about 10 mm. long and 5 mm. at the widest portion. Figure 1 is a photograph of several plants, among them an early- and a late-maturing variety.

The seed is composed of a kernel surrounded by a thick hull or pericarp, primarily fibrous and containing few nutrients; the kernel is rich in oil and protein content. Claassen (7) found, as a general trend, that high nitrogen content in the kernel is associated with a low oil content. In seeds bred for low hull content, however, both high nitrogen and high oil content were possible to attain. Depending on the genetic origin of the plant, the pericarp will vary from 25 to 70% of the entire weight of the seed. It is this variation in percentage of hull,



FIG. 1. Several safflower plants, the one to the left is an early emergence variety.

oil, and nitrogen which is responsible for the differences in analysis of the seed and meal recorded in the literature. For several varieties grown in the United States, oil content varies from 30 to 36%, protein from 16.8 to 10.8%, and hull content from 38 to 45%.

4. Processing

Safflower seed has been processed by continuous screw press and prepress solvent-extraction. The prepress solvent-extraction combination is used because it is more economical than solvent extraction or screw press.

The method of application of the continuous screw-press is quite conventional. Seed is either ground or partially decorticated prior to cooking to a maximum temperature of 240°F. The cooked material handles easily in the screw press but requires a water-cooled shaft and

circulating cooling oil kept at 140°F. in order to reduce the heat generated in pressing such a fibrous seed. It is difficult to measure the maximum temperature in the press, but it is above 240°F. for a very short interval at the highest pressure point.

If the seed is partially decorticated, the hulls are ground separately and mixed in later with the meal on a continuous basis. The fact that some of the hulls are not fed through the press affects the quality of the products only slightly, any change being toward improvement because of generation of less heat. Control of exact fiber and protein levels on partially decorticated meal is impractical.

One important processing consideration is the need for cooling the cake immediately after extrusion. If the cake is ground while hot, sacked, and piled immediately without cooling, oxidation of the oil starts, the first indication of which is the acrid odor of an oxidized drying oil. This is followed by "spontaneous" heating of the meal in the pile. After considerable storage at normal temperature, or for shorter periods at elevated temperature, the fat content begins to decrease. At normal storage temperatures this may take two to three years. Storage at 100°F., however, hastens the reaction in improperly cooled meal so that the oil content may start to decrease within three months. An antioxidant, when properly added, has been found to be effective in stabilizing the residual oil. Well-cooled, whole pressed safflower seed has been stored for more than two years without any adverse effect on quality.

There is no evidence of any need for extra cooking or toasting after oil extraction, as is done with soybean meal, nor is moisture control to avoid caking or fermenting as critical with safflower as with many others.

5. Composition of Meal

Seed produced in California yields, after removal of oil, a product containing 16 to 24% protein and 30 to 37% fiber. Table I (3, 8-11) presents the ranges in composition of typical meals. Factors other than seed variety, such as climate, manner of farming (irrigated versus dry-land farming), and type of soil affect the proportions of protein and fiber.

Nomenclature must be used carefully in order to avoid confusion between the whole pressed seed and the material which is pressed after the hull is removed, i.e., decorticated meal or hulled seed. The Association of American Feed Control Officials (12) has established the nomenclature as follows: whole pressed safflower seed and safflower meal, the latter for the hulled product.

The higher ash content is in the kernel of safflower seed; this is true of some other seeds such as sunflower seed. The result is a higher ash content for the meal than for the whole pressed seed. The ash of whole pressed seed contains the following: 0.9% potassium, 0.74% phosphorus, 0.4% magnesium, 0.5% calcium, and 0.06% sulfur. Sodium, iron, and silicon are present in the range of 0.01 to 0.1%, and other

TABLE I
COMPOSITION OF WHOLE PRESSED SAFFLOWER SEED AND SAFFLOWER MEAL

Source	Dry matter	Crude protein (N \times 6.25)	Fat	Fiber	Ash	Nitrogen-free extract
	%	%	%	%	%	%
University of California ^a						
Whole pressed seed ^b	92.0	19.0	6.0	33.0	4.0	30.0
Decorticated meal ^b	92.0	36.0	7.6	17.5	7.4	23.5
Scharrer and Schreiber ^c	92.2	32.9	9.1	21.6	6.9	21.7
Morrison ^d						
Decorticated seed	91.0	38.0	6.8	21.0	8.2	17.0
Undecorticated, unhulled seed	91.0	18.2	5.5	40.4	2.8	24.1
Rabak ^e	95.9	19.2	5.8	42.6	2.9	15.4
A. Khan, undecorticated seed ^f	91.4	25	8.8	32.3	4.2	21.1

^a H. Goss and K. K. Otagaki, *Calif. Agr.* **8** (5), 15 (1954).

^b A blend of N852, N6, and Indian varieties.

^c K. Scharrer and R. Schreiber, *Biedermanns Zentr. B. Tierernähr.* **14**, 355 (1942).

^d F. B. Morrison, "Feeds and Feeding," 21st ed., p. 1127. Morrison, Ithaca, New York, 1951 (c 1948).

^e F. Rabak, *U. S. Dept. Agr. Circ.* **366** (1935).

^f A. Khan, *Oil & Oilseeds J. (India)* **4** (4), 17 (1951).

trace elements occur at levels less than 0.01%. The fibrous, stiff hull of the safflower seed, which constitutes from 25 to 70% of the seed contains protein 3 to 4%, fat 1 to 4%, fiber 50%, ash 2.0%, and pentosans 28.5%.

The amino acid composition of the protein is given in Table II (13a-13f). These data have been confirmed generally in poultry feeding tests.

The fiber of the safflower seed was the subject of study by Otagaki (14). It is relatively indigestible, although not as much so as the earlier publications (9, 15) would indicate. (See also Schneider, 16, and Morrison, 10.) The earlier conclusions of a negative or zero digestibility were based on a digestion trial of 10 days' duration in which the safflower fiber constituted less than 15% of the total fiber in the ration. More recent work of Goss and Otagaki (8) revealed a coefficient of digestibility of 22 and 23%, as shown in Table III. This

TABLE II
ESSENTIAL AMINO ACID COMPOSITION OF CRUDE PROTEINS IN MINOR OILSEEDS

References	Safflower meal		Almond kernels	Walnut meal		Babassu meal		Hemp-seed	Edestin	Pecan
	a	b	c	a	b	a	b	d	e	f
	%	%	%	%	%	%	%			
Crude protein ^g	22.1	18.0	23.6	13.0	20.3	22.7	24.3	—	—	—
Calculated as grams per 16 g. of nitrogen										
Arginine	7.8	8.5	11.1	7.5	12.1	14.1	14.3	5.0	16.7	10.4
Histidine	2.0	2.1	1.5	1.6	2.1	1.8	1.8	3.9	2.5	3.0
Isoleucine	3.8	4.7	0.4	3.2	3.7	3.9	4.2	4.4	4.7	4.9
Leucine	5.5	7.3		5.3	7.9	6.2	6.8	7.7	7.5	8.2
Lysine	2.7	2.9	6.6	2.2	2.7	4.3	2.9	2.7	2.4	5.4
Methionine	1.5	1.1		1.2	1.0	2.3	1.1	2.2	2.4	1.7
Phenylalanine	5.2	3.7	2.4	3.3	3.5	5.9	3.7	5.8	5.5	5.3
Threonine	2.9	3.5		2.6	3.5	3.2	3.5	3.8	3.9	4.0
Tryptophan	1.2	1.0	1.3	1.5	1.0	1.0	1.0	1.5	1.5	1.4
Valine	4.9	6.0		3.8	5.1	5.3	6.0	6.3	6.5	3.5

^a C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).
^b R. W. Carroll, The Quaker Oats Co., private communication, 1956.
^c California Almond Growers Exchange, private communication.
^d R. J. Block and D. Bolling, "The Amino Acid Composition of Proteins and Foods," 2nd ed. Charles C Thomas, Springfield, Illinois, 1947.
^e G. R. Tristram, in "The Proteins" (H. Neurath and K. Bailey, eds.), Vol. 1, Part A, p. 217. Academic Press, New York, 1953.
^f C. H. Edwards, L. P. Carter, and C. E. Outland, *J. Agr. Food Chem.* **3**, 952 (1955).
^g Calculated as nitrogen × 6.25.

TABLE III
TOTAL DIGESTIBLE NUTRIENTS IN SAFFLOWER MEAL^a

Nutrient	Composition		Coefficient of digestion	
	Undecorticated	Decorticated	Undecorticated	Decorticated
	%	%	%	%
Crude protein ^b	19.8	36.0	80	88
Fat	7.6	7.6	82	89
Fiber	32.7	11.5	22	23
Nitrogen-free extract	27.4	23.5	49	63
Total digestible nutrients	50.4	66		
Digestible protein	15.2	32		

^a According to H. Goss and K. K. Otagaki, *Calif. Agr.* **8** (5), 15 (1954).
^b Calculated as nitrogen × 6.25

was based on a three month digestion trial in which the safflower fiber was fed at 25, 50, and 75% levels. Apparently the high lignin content of the hulls, 25%, may bind the cellulose chemically and reduce its availability to the ruminant. Otagaki (14) tried treatment of the fiber with numerous chemical reagents to increase its digestibility and was able to obtain 50% digestibility after sodium hypochlorite treatment.

6. Uses of Meal

a. Whole Pressed Safflower Seed

(1) *Dairy cattle.* Mead *et al.* (17) reported that whole pressed safflower seed, though not by itself palatable to dairy cows, is readily consumed when added to palatable concentrates in an amount equal to 25% of the total mixture. Milk samples collected prior to and during an intermittent feeding period of 27 days failed to reveal any objectionable flavors attributable to the safflower product, even though several cows at single feedings consumed as much as 5 pounds of the safflower concentrate prior to milking. Other investigators reported that whole pressed safflower seed containing 20% protein may be used in a grain ration for milk cows fed liberal amounts of good quality alfalfa hay (18) or can be substituted for linseed meal in a dairy ration (19). It has neither laxative nor constipating effects.

In California, the primary use of whole pressed safflower seed is in dairy cattle rations; over 26,000 tons were used for this purpose in 1953 and 1954. When fed correctly, both the quality and quantity of milk production are maintained. The average dairy feed mixer adds 10 to 15% to the ration, although a few large-scale mixers have added 20%. It has been used to replace, to some extent, both barley and the high protein oilseed meals. It is also utilized to a considerable extent merely as an addition or extender to the whole ration without replacing any particular constituent.

(2) *Beef cattle.* Substantial quantities of whole pressed safflower seed are used in California for feeding beef cattle. Examples of such rations in which whole pressed safflower seed was fed as a supplement to corn silage for growing calves are given in Table IV (20). In this test, the supplements were fed to provide the same level of supplemental nitrogen intake, except for one which had half as much. The safflower pellets contained 24% protein and 33% fiber.

When an equal amount of protein supplement was added to a ration containing barley, dried molasses, beet pulp, corn silage, and alfalfa there was no significant difference in the results when cull peas, whole pressed safflower seed (21.8% protein), or cottonseed meal was the supplement (21). The steers fed the safflower seed product had equally

good a finish as those receiving cull peas or cottonseed meal. Some difficulty was encountered in this trial in obtaining adequate feed consumption unless the whole pressed safflower seed was mixed with the concentrate mixture. This is in line with the experience of many feeders of both dairy and beef cattle. Those who have had difficulty with consumption have not mixed the feed properly or have introduced too much whole pressed safflower seed into the ration at one time.

TABLE IV
WHOLE PRESSED SAFFLOWER SEED FED TO BEEF CATTLE: WEIGHT GAIN AND FEED CONSUMPTION^a

Feed	Pounds per day				
Corn silage	25.6	23.3	24.1	24.4	24.8
Ground shelled corn	4.0	4.0	4.0	4.0	4.0
Soybean oil meal pellets	1.1	—	—	—	—
Whole pressed safflower seed pellets (24% protein-33% fiber)	—	2.0	2.0	1.0	—
Pellets (95% whole pressed safflower seed and 5% urea)	—	—	—	—	1.4

Growth	Pounds				
Average initial weight	452	446	449	450	453
Average final weight	728	706	710	693	720
Average daily gain per head	1.99	1.87	1.88	1.75	1.92

Average feed required for 100 pounds gain	Pounds				
Corn silage	1291	1246	1285	1397	1292
Ground shelled corn	201	214	213	228	208
Supplement	55	107	107	57	75

^a According to M. L. Baker, G. N. Baker, C. Ervin, L. C. Harris, and M. A. Alexander *Nebraska Agr. Expt. Sta. Bull.* 402 (1951).

Safflower hulls were included in a fattening ration for steers as three-fourths of the roughage and almost half of the total ration (22). At this level, the cattle did poorly. At lower levels in the ration the lesser gains are compensated, in part, by the low cost of hulls.

(3) *Sheep*. Weight gains of 0.32 to 0.35 pound per head per day were reported for fattening lambs (23) in a trial in which soybean meal and whole pressed safflower seed were compared two ways, hand-fed and self-fed. The weight gains were similar, and the carcass grades were about the same. By using prices prevailing at the time of the experiment, it was calculated that the safflower product was more

expensive in terms of cost of pounds of gain; however, prices in 1955 would definitely provide a lower cost of gain.

b. Safflower Meal for Poultry

Pure safflower meats (hulls removed) should yield a meal containing 60% crude protein. Because of the similarity in particle size of meats and hulls, however, the practical range of protein content achieved is closer to 40%.

Kratzer fed safflower meal as the sole source of protein to chicks (24). Slight deficiencies of arginine, lysine, methionine, and either or both of glycine and cystine were evidenced. Later work (25) with practical diets showed a deficiency of lysine and methionine in safflower meal-soybean meal combinations. Best results were obtained when equal parts of safflower meal and fishmeal were given. A ration containing 5% fishmeal, 9% soybean meal, and 15% safflower meal also gave satisfactory growth results.

Grau and Zweigart (26) substituted safflower meal, partially and completely, for soybean meal in diets for laying hens and found no significant differences in the rates of egg production. There was no difference in the yolk or albumen conditions in the eggs laid from all groups. After a storage period of six months, the eggs from hens fed safflower meal were as good in quality as those from hens fed soybean meal.

c. Miscellaneous Uses

Because safflower is still a new oilseed crop and the by-product meal and cake are just gaining in stature, uses other than as feeds for cattle, sheep, and poultry have not been developed. A few people have fed the meal to chinchillas and rabbits with good results. Some work was done with turkey laying rations where it was shown that whole pressed safflower seed was helpful in controlling weight. The whole seed has been fed by Draper (27) to livestock and poultry; when ground with other feeds it has a dust-reducing action.

7. Trends

There are several opportunities through research to influence the future of this oilseed crop. By far the most important is agronomic research. New varieties with higher protein and lower fiber content would make the product much more interesting to the feed and oilseed processing industry. Another opportunity to improve the product would be by controlled, fairly complete decortication of the seed. If this were possible, and an economic use of the hulls found, the end-use pattern

would be considerably different. It is now possible to screen out a higher protein fraction, but the economics of such a separation process are questionable. The trend toward solvent extraction or prepress solvent extraction, so evident in soybean and cottonseed processing, is sure to affect safflower processing by providing a means for increasing the yield of oil.

The reason for the unusually rapid decrease in apparent oil content compared with similar meals during high-temperature storage should be investigated. Also, the effect of variations in processing conditions on protein quality should be studied. Because it may be impractical to reduce the fiber through decortication, another solution to improve the utilization of the meal would be to treat it so as to increase the digestibility of the fiber.

III. ALMONDS

1. History

The almond tree, which is of obscure, ancient origin, is widely grown for the flavorsome nut. There are two main growing areas, the countries surrounding the Mediterranean, and California in the United States. Since the primary market for the nut is as a food for direct consumption, there is relatively little meal available. Some oil is produced in England, Germany, and France from nuts grown in such countries as Morocco, Iran, Portugal, Sicily, and Syria (28). Almonds were first introduced in the eastern United States in the 1840's. Although their growth was widespread, the need for improved varieties became evident. By 1910 a statewide growers' cooperative was formed in California to handle marketing and distribution problems and to coordinate information on European production. Throughout the first half of the century growth was steady, until by 1951 the total bearing acreage in California was 90,874 (29).

Any material available for crushing, either as culls and low-grade by-products or from government surplus programs, is of value primarily for the oil. The oil, after refining, bleaching, and deodorizing, goes into the cosmetic and pharmaceutical trade. Its volume is very small compared to that of other oils.

2. Production and Trade

The price of almond kernels in the market for human consumption is in the range of 60 to 80 cents per pound. Oil crushers cannot afford to pay more than 10 cents per pound for the same material; hence every effort is made to meet the quality requirements of the higher-priced market. The study by Adams (30) in 1953 of sixteen average

areas in California, excluding poorly producing farms, showed an average cost of 23.3 cents per pound of almonds in the shell at the hulling point. The average yield during 1948-52 was 818 pounds per acre, with a total statewide production of 39,100 tons (in the shell) per year.

In the 1949-50 and 1952-53 seasons a total of 1700 tons of almonds, kernel weight basis, was released under the commodity subsidy program for oil production in California. When one considers that most screw-press plants will process a minimum of 80 to 100 tons per day, the surplus from two seasons resulted in only enough for 17 to 21 days of processing.

In 1954 Spain and Italy produced 52,000 tons of almonds, and in 1955, 44,000 tons (31, 31a).

3. Nut

The almond tree, *Prunus amygdalus*, is frequently compared to a peach, and, in fact, the fruit resembles a poorly developed hairy peach. The fruit at maturity is not edible except for the innermost kernel, which is analogous to the stone of a peach or apricot. The mesocarp at maturity is thin and tough (32).

Almond nuts are 50 to 65% kernel containing 50 to 60% oil, and 35 to 50% shell. The composition of almonds is given in Table V (33); the amino acid composition of the protein is given in Table II.

4. Processing

The meal of most consistent volume is that derived from culls and low-grade material. In the United States, continuous screw-pressing is the normal process because there is insufficient material to process in a solvent-extraction plant. The almond culls offer a difficult raw material because of their variable shell content. They are not cleaned but are processed directly in a conventional screw-press operation, after cooking up to 240°F. After screw-pressing the meal is cooled and ground.

Whenever almond kernels are available, they are crushed in much the same fashion as sesame seed. It is easier to crush the kernels than the culls, since the kernels are more consistent in quality and have a lesser amount of shell; neither is there as much heat generated when processing the kernels.

5. Meal—Composition and Digestibility

Almonds crushed as a normal oilseed yield a meal containing approximately 5% fat, 38 to 48% protein, 5.5% fiber, and 6% ash.

TABLE V
COMPOSITION OF ALMOND, WALNUT, AND PECAN KERNELS^a

Component	Almonds	Walnuts	Pecans
	<u>%</u>	<u>%</u>	<u>%</u>
Moisture	4.7	3.3	3.0
Crude protein ^b	18.6	15.0	9.4
Fat	54.1	64.4	73.0
Total carbohydrate	19.6	15.6	13.0
Crude fiber	2.7	2.1	2.2
Ash	3.0	1.7	1.6
Calcium	0.25	0.08	0.07
Phosphorus	0.48	0.38	0.32
Iron	0.004	0.002	0.002
	<u>mg./100 g.</u>	<u>mg./100 g.</u>	<u>mg./100 g.</u>
Thiamine	0.25	0.48	0.72
Riboflavin	0.67	0.13	0.11
Niacin	4.6	1.2	0.9
Ascorbic acid	Trace	3	2
	<u>Cal./100 g.</u>	<u>Cal./100 g.</u>	<u>Cal./100 g.</u>
Energy	597	654	696

^a According to B. K. Watt and A. L. Merrill, *U. S. Dept. Agr., Agr. Handbook* 9, 16 (1950).

^b Calculated as nitrogen × 6.25.

The cleanings, culls, and waste nuts after removal of oil are usually called almond screenings feed or almond screenings oil meal and contain 10 to 11% protein, 5 to 6% fat, 22 to 25% fiber, and 6 to 7% ash.

Barre (34) found at least five protein fractions in a protein extract of almond. He was able to separate an albumin, a globulin, and emulsin fraction. This latter fraction has both phosphatase and glucosidase activity. There was considerable phytic acid present, bound to the protein. Spies *et al.* (35), following the procedure for isolating the principal allergen of cottonseed, found typical natural proteoses possessing similar allergenic and other antigenic properties in a wide variety of nuts and other oil-bearing materials. Rocchetti (36) found cyanogenetic amygdalin in almond meal. This was eliminated by cooking the cakes until the glucosides were hydrolyzed completely. The almond cake was useful for feeding to domestic animals when given in amounts within ordinary forage ratios.

Morgan *et al.* (37) reported that meal prepared in the laboratory from kernels contained 47.9% protein, 4.3% fat, 6.4% ash, 10.7% water, 4.2% fiber, 30.6% total carbohydrate, 8.5% sucrose and easily

hydrolyzable sugars, 7.6% pentosans, and less than 1% free reducing sugar. The digestibility of almond proteins was found to be 94% of that of beef proteins (38).

6. Uses

The primary use for almonds is for direct edible consumption, distributed either in the shell or as shelled almonds, blanched almonds, roasted, toasted, french-fried, or sugared. Many almond products are made by grinding the almonds, cooking or roasting, and adding other ingredients such as sugar, salt, or water. One of these, almond paste, used in the baking and candy trade, is made of blanched kernels which are ground, cooked, and mixed with sugar and water and added flavoring (39). A product known as fine almond meal and medium almond meal, produced from the slicing and dicing machine operations, is available in the United States in small tonnages and is used by the bakery trade for topping. There is a larger demand for the various almond meal products for home baking in Europe, and a substantial volume moves to this market.

The culls and low-grade almonds, which have no use as a food, are crushed by the oil mills located near the almond-producing centers. These materials usually contain a high proportion of shells, thereby yielding a low-quality meal compared to that from pure kernels. Almond screenings feed or almond screenings oil meal is sold either to the dairy feed mixers or cattle feeders. One of the greatest difficulties is the intermittent supply which does not allow a feed mixer to maintain inventories and keep the item on his meal tags. Because the volume is so small, most of the material moves directly to a consumer.

7. Hulls

The largest usage of an almond by-product in the feed industry is the consumption of almond hulls by beef and dairy cattle. Almond hulls, the outer portion of the almond, are freed from the almond by drying and hulling; the shell with inside kernel is then sent to the shelling plants. The total digestible nutrient material of the hull is 72%. It is available in the United States in quantities of the order of magnitude of 50,000 tons per year at a cost in 1956 of \$30 to \$36 per ton.

8. Trends

The long-term trend in the United States has been to reduce imports of almonds and concentrate on the more profitable growing areas in

California. The supply of almond kernel meal for animal feed depends on the government support programs but will not be available as a rule. The almond screenings meal will continue to be available in local areas in very small volume. Almond kernels for human food will continue to be an excellent source of nutrients and will grow in amount produced with the popular demand.

IV. WALNUT

1. History

The walnut's origin is obscure because it is older than recorded history. The so-called English walnut is the Persian walnut which was sent to England and grown there for many years. On a world-wide basis, the Persian walnut is of greater importance as a food crop than any other nut tree grown outside the Tropics (40). In 1867 the first walnuts were planted in Southern California, probably from nuts imported from Chile. In 1870 many French varieties were started in Northern California, which has since become the major producing area in the United States (41).

Of course, the walnut is grown for human consumption. Any by-products processed in an oil mill represent an exceedingly small proportion of the total tonnage. The oil obtained from walnuts is used both as an edible and as a paint oil.

2. Production and Trade

The average annual production of unshelled walnuts in California for the period 1945-54 was 65,000 tons (23,646 tons shelled to yield 8575 tons of walnut meats) (42). Oregon's production averaged 7320 tons of unshelled walnuts. There is no commercial production elsewhere in the United States. The annual average production (1948-52) of Persian and English walnuts in other countries is as follows: France 17,300; Italy 22,500; Turkey 7900; and Yugoslavia 4100 tons unshelled basis (43). Although walnuts are widely grown, there is an active export trade in both shelled and unshelled walnuts from India, China, Japan, Iran, Syria, Roumania, and Chile.

In 1949, 1952, and 1954, walnuts were diverted under the U. S. Government surplus program to oil mills for crushing. In these years, 12,120, 4300, and 500 tons were diverted. In 1949, 1050 tons and in 1952, 1430 tons of low-grade culls were crushed. Because of the variation in composition of culls, it is difficult to estimate the yield of meal produced. It is evident, however, that the amount of walnut meal produced is very small compared to most other vegetable oil meals. Meal

prices vary with the quality, and the prices of competing feeds. Because of the variability in volume and availability, price quotations are meaningless.

3. Nut

The walnut is of the genus *Juglans*; the Persian or English species is *Juglans regia*. The shell, or endocarp, is extremely hard and consists of dense stone tissue with a thin lining of brown parenchyma. Inside the shell are the meats, fleshy, much wrinkled, two-lobed cotyledons. Small aleurone grains, less than 10 μ in size, and fat are the visible cell contents in the cotyledons (32).

English walnut kernels have the composition given in Table V. Oil and protein content were found to be independent of the size of the walnut but related to each other, higher oil content being accompanied by a lower protein content (44).

4. Processing

Variability in volume and quality of supply makes the processing of walnuts difficult. The mechanical screw press is used in the United States; temperatures and processing conditions are similar to those used for safflower. The hard shell aids in the pressing of the oil, but it also causes excessive wear and tear on equipment. Variation in shell content has to be compensated for in processing by different settings on the machines which expel the oil.

5. Meal—Composition and Nutritive Value

As with the other by-products from tree nut sources, walnut meal varies considerably in composition, depending largely on the amount of shell, and to some extent on variety. If clean kernels (shell-free) were crushed, the resultant meal normally would comprise 39.5% protein, 5% fat, 5.5% fiber, 4.5% ash, and 10% moisture. It is impossible, however, to remove all the shells economically; actual analysis of normal production from a major crusher of walnut by-product is given in Table VI.

Honcamp *et al.* (45) analyzed and conducted digestibility trials on the cake from both shelled and unshelled walnuts, with results as given in Table VI. (The variety of nuts was not mentioned, although they were of a much higher protein type than normal.) Total digestible nutrients calculated from Honcamp's data are 94% for the shelled walnuts and 62% for the unshelled walnut cake. It should be pointed out, however, that the trial was run with a ration consisting of 600

TABLE VI
WALNUT MEAL: PROXIMATE CHEMICAL COMPOSITION AND DIGESTIBLE NUTRIENTS

Material	Crude protein (N × 6.25)	Fat	Fiber	Nitrogen- free extract	Ash
	%	%	%	%	%
Average-protein commercial meal ^a	13.6	6.3	25.0	44.3	3.3
High-protein commercial meal ^a	31.1	7.8	19.6	29.7	3.8
Cake from shelled walnuts ^b	45.8	9.6	6.7	32.1	5.7
Cake from unshelled walnuts ^b	23.2	9.0	30.9	31.9	5.0
Coefficient of digestion for cake from shelled walnuts ^b	91.3	99	37	95	
Coefficient of digestion for cake from unshelled walnuts ^b	84	96	15	59	

^a Pacific Nut Oil Co., private communication.

^b F. Honcamp, H. Zimmermann, and E. Blanck, *Landwirtsch. Vers. Sta.* **93**, 77 (1919).

parts of a meadow hay and 250 parts of walnut cake. The walnut nutrients formed a relatively small part of the ration; a higher percentage of walnut cake might have given different results.

Mignon (46) obtained normal growth with the total proteins of the walnut fed at a 12% level of protein. Cajori (47) also found good growth in rats fed walnut proteins. With beef protein rated as 100% in digestibility, the walnut protein had a digestibility of 84% (38).

6. Uses of Meal

Because walnut meal is available in relatively small amounts and of widely varying quality, it is not found as a regular ingredient on feed mixers' tags. The beef cattle feeders are more suited to take into account the normal variations and high fiber content of the meal, since their operations consume the material directly.

There is one most unusual usage of walnut meal worth mentioning. A small industry which grows earthworms for sale finds that walnut meal serves as an excellent feed for the worms. Naturally the tonnage is rather small.

7. Trends

A trend is difficult to observe because of the very small volume of by-product meal from the walnut cleaning operations. A by-product industry of this size cannot bear the costs of research to develop properly the optimum markets.

V. BABASSU

1. History

Babassu, one of the few wild-growing palms which yields an oil-bearing nut collected in sufficient quantities, was at one time considered an item of international commerce. The babassu palm is primarily Brazilian, the only export on record having been from Brazil. Although the number of trees is large, the yield of fruit from these trees is limited by a number of factors. Weather and transportation conditions limit economical collection to the Brazilian states of Maranhão and Piauí (48). Another limitation is the variable availability of labor for collecting and cracking the babassu nuts, which is the most difficult phase of the whole industry. Many attempts have been made to develop machinery for cracking the nuts, but all have been unsuccessful, so that hand labor is still necessary.

The first commercial shipments of the kernels were made by Brazil to Europe in 1911. Although United States entered the import market in 1935, exports from Brazil were negligible after 1951. Under reciprocal trade agreements between Brazil and the United States, babassu kernels, oil, or meal enter the United States duty free.

Babassu oil is used for soap and edible purposes.

2. Production and Trade

The babassu tree may bear up to 55 to 110 pounds of kernels per year. From Brazilian statistics, Markley (49) has found that during 1940-55, production of the kernels averaged 66,000 metric tons per year, of which an average of 24,000 tons was exported annually in the period from 1940 to 1951. Exports have dwindled since then, owing to an increasing domestic demand. The production is amazingly large for a hand-cracking operation.

Babassu meal prices are necessarily tied to copra meal prices. Although its protein content is slightly higher, the price of babassu meal is generally \$3.00 to \$5.00 lower per ton than copra meal; the lower price is due to the requirements for extra tagging, additional inventory, and other costs of a small-volume commodity. In the years 1950-55 the price ranged from \$60 to \$80 per ton in California. At this price level, the meal is purchased more as a source of total digestible nutrients than as a protein concentrate.

3. Nut

The babassu palm, *Orbignia speciosa*, is a member of the *Palmae*. It grows to a maximum of 50 to 60 feet in height, with a trunk 2 feet in

diameter. The average density of growth of mature trees is 60 trees per acre. After flowering, the palm bears two to four clusters of about 250 nuts each, twice a year. Each nut consists of an extremely hard shell containing two to six kernels held by a hard fibrous mesocarp. The kernels vary greatly in size, but approximate $1\frac{1}{2}$ inches long and $\frac{1}{2}$ inch in diameter, are surrounded by a thin brown skin, and are white and of hard, brittle consistency. The percentage, by weight, of kernel in the whole fruit varies from 1 to 15% with an average of 6%; the remaining 94% has relatively little value except as fuel (49). The kernel ranges from 64 to 70% in oil content; the constituents of the oil are almost identical with those of coconut oil. The amino acid composition of the protein is given in Table II.

4. Processing

The babassu kernel is normally processed by the continuous mechanical screw press. As an oil-bearing material it rates high in regard to ease of handling in normal oil mill equipment. The kernels are first screened to remove any foreign material, then ground on a high-speed hammer mill. Conventional cooking is at temperatures of 220 to 240°F. Because of the high oil content, greater allowance than usual has to be made in setting the spacing through which the oil is squeezed. A continuous flow of cooling oil at 150°F. is sufficient to obtain a light-colored oil and a good, light cake. The cake is cooled and then ground prior to sacking. There are no problems in storing and handling babassu meal which is usually handled in burlap sacks each containing 100 pounds.

5. Meal—Composition and Digestibility

Commercial babassu meal, as made in the continuous screw press, will contain 22 to 25% protein, 5 to 6% fat, 12 to 15% fiber, and 5 to 6% ash. It has a pleasant odor and is somewhat lighter in color than copra meal, because the babassu nut is not dried artificially with direct fire as usually is copra. Its total digestible nutrients range from 72 to 82%.

Honcamp and Petermann (50) studied the digestibility of a babassu meal which contained 24.9% protein, 6.79% fat, 15.0% fiber, 5.89% ash, and 47.39% nitrogen-free extract. Using 500 parts of clover hay and 200 parts of babassu meal, they fed sheep for a 10-day period to determine the coefficients of digestibility. The digestibilities of the nutrients were protein 85%, fat 98%, fiber 18%, and nitrogen-free extract 71%. Folger (51) found a much higher digestibility for the

fiber, 71%. This figure should be more accurate, because Folger used a higher percentage of babassu meal. He noted that the babassu meal was quite palatable; although too large a proportion of the meal in a ration caused some scouring. The laxative property may have been due to a high magnesium content of 0.97%. The calcium content was 0.13%, and that of phosphorus, 0.49%.

6. Uses

In California, babassu meal is used exactly as is copra meal because of its similar analysis, appearance, and odor. Almost all the babassu meal goes into mixed dairy feeds because of its high palatability, digestibility, and preference of the dairyman for this type of feed. Its sporadic availability is a disadvantage; since the dairy feed mixer must print each ingredient on the tag identifying the mixed feed, he prefers material which is always available.

7. Trends

The trend in Brazil, the sole source of the babassu, is toward increased domestic consumption of products such as soap, margarine, and cooking fats from babassu oil. Consequently the percentage crushed in Brazil is increasing. The greatest single factor which would change the trend of the babassu industry would be the design of an efficient, economical machine to crack the nuts near the collection centers in the Brazilian jungles and plantations. A great deal of money and effort has been spent on such a machine without success thus far. Additionally, the very low yield of nuts may make such an operation impractical unless agronomic research develops a babassu fruit with a higher kernel content.

VI. HEMPSEED

Hempseed, *Cannabis sativa*, is a plant of many varieties. Some varieties, although called hemp, are not botanically related to the true hemp. Hemp is grown for fiber and seed, but the fiber crop is cultivated in areas separate from the seed crop because of the difference in growing conditions. The average production in the Soviet Union is 250,000 tons, in Manchuria about one-half of this. In Chile the production ranges from 4000 to 18,000 tons, and in Turkey 4200 tons of seed were produced in 1955 (52). The oilseed crop is also raised in Formosa and Roumania. International trade has dwindled to a few thousand tons, since Manchuria was the primary exporter of hempseed; the production of the Soviet Union is consumed internally and, therefore, does

not enter foreign markets. Because there is relatively little seed available, there is not much known about the meal.

The best available work on digestibility of hempseed meal is that of Folger (51) (Table VII). His work was on two lots: the first (lot 1) was light brown, finely ground, and overheated in the screw press; the second (lot 2) was coarsely ground and dark green. The second lot was obviously more palatable to the sheep than the first; however, there was no difference in the digestion coefficients of the two lots, as is shown in Table VII. The unusual quality of hempseed meal is its low coefficient of digestibility of the fiber and nitrogen-free extract. Because of the resultant low total digestible nutrients, the price of this

TABLE VII
PROXIMATE COMPOSITION OF HEMPSEED MEAL^a

Component	Composition		Coefficient of digestion
	Lot 1	Lot 2	
	%	%	%
Solids	93	91	44
Crude protein ^b	31	30	84
Fat	5	7	85
Fiber	23	24	12
Nitrogen-free extract	24	20	14

^a According to A. H. Folger, *Calif. Agr. Expt. Sta. Bull.* 604 (1937).

^b Calculated as nitrogen \times 6.25.

material would be also relatively low. The two lots contained, on the average, 0.25% calcium, 0.43% phosphorus, and 0.78% magnesium.

Hammond (53) found that as much as 15% of ground hempseed meal could replace satisfactorily an equal weight of soybean meal in rations for growing chicks containing originally 35% soybean meal. The primary usage for hempseed meal in the United States, when it was available, was in beef and dairy cattle rations.

One of the interesting protein components of hempseed is edestin, a globulin. This plant protein is one of the more extensively studied, principally because of its ease of purification and crystallization (13e). Goring and Johnson (54) report that the crystalline product is not monodisperse but that a monodisperse fraction can be prepared by suitable fractionation procedures. They suggest that this edestin preparation can be used as a primary protein standard.

Because the largest volume of hempseed is grown in the Soviet Union and Manchuria, there has been very little world trade in this

commodity. Not until trade channels open up again will there be any wide-spread interest in hempseed meal. If the hempseed should become available again, it would be interesting to determine what effect new processing methods would have on the digestibility and nutritive value of the meal.

VII. PECANS

Pecans have been grown for years for human consumption. As in other nut industries, the culls and nuts useless for human consumption are crushed for the oil and by-product meal. The production of pecans annually in the United States averaged 70,000 tons in the period 1944-53 (42). The production is centered in the Southern states, with Oklahoma and Georgia as the leading areas. The Oklahoma pecans contain from 45 to 60% meats with an average oil content of 60 to 73% (55).

The protein of the pecan has been investigated by Mitchell and Beadles (38) and compared to beef protein; it was found to be 71% as digestible. The chemical composition of the fresh pecan is shown in Table V; its amino acid composition is given in Table II.

Because of the small amount of by-product or waste material available, there is little material in commercial channels which does not go directly into human consumption. With high prices prevailing for the pecans, it is doubtful if there will be any significant use of this protein source for animal consumption. There is a small industry, based on the availability of about one million pounds annually of culls, which produces pecan oil for the cosmetic industry and tannin for leather manufacture and oil drilling (56).

VIII. ILLIPÉ NUT (57)

The Borneo illipé nut, *Shorea stenoptera*, is processed for its hard oil (Borneo tallow), and the meal finds a place in feed formulation. The trees, depending on the type, grow to heights of 20 to 60 feet, with trunk diameters of $1\frac{1}{2}$ to 3 feet. The nuts are distinguished by size, color, and port or district of origin. Large types after decortication measure about 2 inches long by $1\frac{1}{2}$ inches in diameter and weigh up to 30 g. each. The small type appears to grow in commercial quantities only in the northern and western districts of the islands, i.e., in Sarawak and Pontianok, whereas the larger varieties are widely distributed.

World trade fluctuates widely because of uncertainties of harvest; estimated world trade was 3500 long tons in 1953 and 30,000 tons in 1954. A little over half of this tonnage is imported to the United Kingdom.

The kernels are enclosed in a thin brittle case which sits in an acorn-like cup with spinneret, wing-like attachments to enable the seed to be carried clear of the parent tree. In the case of the large varieties, these spinnerets or vanes, which are five in number, measure up to 6 inches long, the whole undecorticated seed being 8 to 9 inches over-all and weighing 60 g. It can be imagined that a considerable fall of such seeds, resembling oversized shuttlecocks, from a height of 60 feet is quite an event.

The trees grow on the low-lying savannas along the rivers which are flooded during the rainy season, June to August, this being the period of the southwest monsoon. They are in bloom just before the onset of the northeast monsoon, and if, as often happens, the break of the monsoon occurs early and

TABLE VIII
PROXIMATE COMPOSITION OF ILLIPE NUT MEAL^a

Component	Type of nut	
	Black	Red-brown
	%	%
Moisture	10.2	10.9
Oil	2.8	1.8
Crude protein ^b	15.7	10.4
Ash	2.0	3.4
Sand	2.0	0.2
Indigestible fiber	6.1	4.1

^a J. G. Collingwood, Unilever Ltd., London, 1956, private communication.

^b Calculated as nitrogen \times 6.25.

with very heavy storms of wind and rain, the blossom and immature seeds are stripped from the trees and the crop is not worth collecting. This set of circumstances occurs so frequently that it has given rise to the belief that the trees fruit only once in five years. Yield of seed in a good season is large and has amounted to 30,000 tons actually shipped, with the possibility of as much again remaining uncollected. There have been occasions in some localities where in times of flood the tributary streams have been blocked by seed floated off the land.

The nuts are collected from the ground and dried in the sun until the shells are sufficiently brittle to be separated from the kernels by pounding in rice mortars and hand picking. When sufficient seed has been gathered and decorticated it is transported down river by canoe and bartered to the first trader.

The seed is almost invariably solvent-extracted in batch plants after breaking, cooking with live steam to a temperature of 110°F., and flaking. Desolventization is with live steam up to 105°F., and the re-

sultant wet meal is discharged from extractors to dryers where the moisture is reduced to approximately 12%. Proximate composition of the meal is given in Table VIII.

The meal from the extracted *Shorea stenoptera* has a low crude protein content and is not an important feedstuff. It is, however, non-toxic, has some feeding value, and is able to find a place in some formulations where a cheap source of carbohydrate or low protein is required, its market value being about two-fifths that of undecorticated cottonseed meal.

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CHAPTER 23

COCONUT OIL MEAL

LEO V. CURTIN

I. INTRODUCTION

Coconut oil meal is obtained after extraction of the oil from the kernels of nuts of the coconut palm, *Cocus nucifera*. The coconut and coconut oil have been produced and widely used in the tropics and subtropics from the earliest times. During the present century, coconut oil has come into extensive use in Europe and America.

The coconut is now one of the most important oil-bearing products to enter into world trade. Although fresh coconuts are used extensively in countries where they are grown, they enter into world trade only to a limited extent. The important products of commerce are dried coconut meats, or copra, and coconut oil which has been expressed from copra. Coconut oil is the most valuable commodity produced from the coconut. In 1954 the total world production of coconut oil ranked second among the vegetable oils (1). World production of soybean oil exceeded coconut oil by less than 1%. In world trade, however, coconut oil, either as oil or in the form of copra for oil extraction, far exceeds the next ranking oil (2).

The oil is removed from the copra in processing, and the remaining residue, which contains about 20% crude protein and a small amount of oil, is ground into meal and used for animal feeding. This meal is referred to as coconut oil meal or copra oil meal. Since copra contains about 65% oil, its economic importance is for the oil. If the value for oil is assumed to be five times that of oil meal, coconut oil meal is worth only 11 cents for each \$1.00 value of the oil produced from a ton of copra.

The practice of feeding coconut oil meal first became widespread in the European countries. Now, however, coconut oil meal is known in all sections of the world as an excellent source of nutrients for livestock. Where available, it is in great demand for dairy cattle feeding.

Because of its relatively low value per pound compared with coconut oil, coconut oil meal does not enter into world trade to any great extent.

II. PRODUCTION AND TRADE

1. Production of Copra

It is difficult to obtain accurate statistics on world production of copra and coconut oil, for the data are inadequate in many of the copra-producing countries. Although it is possible to obtain reliable data on the amount of copra that enters into world trade, large quantities of coconuts are produced and used in some of the tropical countries without any record of the acreages grown or the amount produced. Gothwaite wrote, in 1925, "The coconut palm is the most important item in the domestic economy of the natives of the tropical belt all around the world, and especially in the East Indies and Malaysia. From time immemorial it has furnished them food and drink, shelter and clothing, weapons and utensils" (3). This same situation still exists today, over thirty years later. The best index on copra and coconut oil production is gained by a review of the export trade.

a. Areas of Production

Coconut palms grow in the tropical belt, extending between 20° and 25° north and south of the Equator. They are grown in greatest abundance in southeastern Asia and Oceania. Prior to the war, Indonesia was the most important producing area. In the postwar years, the Philippine Islands have replaced Indonesia as the leading copra-producing area. Mexico is the most important producer of copra in the Western Hemisphere. In Africa, Mozambique is the principal producer of copra.

b. Volume of Production

The estimated production of copra by country is given in Table I.

The statistics for the years 1938 and 1946-54 were taken from data compiled by the Intelligence Branch of the Commonwealth Economic Committee in London (4). The United States Department of Agriculture estimates of world production are also given at the bottom of the table (1). These data, which were calculated from coconut oil production data (1) based on 63% oil yield from copra, are somewhat higher than the Commonwealth Economic Committee estimates. A recent publication by the Food and Agriculture Organization of the United Nations also gives data on world production of copra for the years 1948-53 (5). These figures are similar to those compiled by the Intelligence Branch of the Commonwealth Economic Committee. World production was low during the war years but recovered quite rapidly

in 1946 and 1947, reaching a postwar peak in 1951. Production was lower in 1952 and 1953, owing to poor climatic conditions in the Philippines, Indonesia, and Ceylon.

The Philippine Republic and Indonesia produce about 60% of the world output of copra. Indonesia was the leading world producer of copra in pre-World War II years but has not retained that position since the war. Production in Indonesia since the war has not reached

TABLE I
ESTIMATED WORLD PRODUCTION OF COPRA^a

Country	1938	1946	1947	1948	1949	1950	1951	1952	1953	1954
1000 short tons										
Asia										
Philippines	767	224	1098	974	767	862	1142	1052	943	1112
Indonesia	913	314	459	672	784	750	896	739	818	851
Ceylon	261	168	158	248	236	213	274	291	261	213
India	179	157	179	157	190	199	203	207	197	202
British Malaya	202	34	78	120	138	168	179	172	170	184
British Borneo	22	8	11	18	29	28	29	28	30	29
Indo-China	32	22	19	18	17	18	19	19	17	18
Southwest Pacific										
New Guinea and Papua	96	19	22	34	48	69	78	84	90	95
Fiji	39	31	38	39	37	31	39	45	37	43
New Hebrides	13	11	22	24	25	25	30	25	27	25
French Oceania	29	28	29	32	30	26	30	28	22	25
Other British Pacific Islands	62	34	56	45	62	63	67	67	62	67
Africa										
Mozambique	45	67	40	59	59	53	48	53	54	45
Tanganyika	12	10	6	9	18	12	10	17	13	15
Western Hemisphere										
Mexico	31	35	30	30	35	50	54	55	66	83
Trinidad	7	20	20	13	16	16	18	24	17	18
Other countries	130	102	112	119	134	136	144	143	175	105
World total ^c	2841	1284	2377	2611	2625	2719	3260	3049	2999	3130
Total ^b	Average 1935-39		Average 1945-49							
	3397		2587		3587		3270		3191	

^a U. K. Commonwealth Economic Committee, "Vegetable Oils and Oilseeds." H. M. Stationery Office, London, 1953, 1954, and 1956.

^b According to estimates of the *U. S. Dept. Agr. Foreign Crops and Markets* 70 (5), 118 (1955).

^c This value is approximately 3340 for 1955, U. K. Commonwealth Economic Committee, "Vegetable Oils and Oilseeds." H. M. Stationery Office, London, 1957.

the 1938 level of production, but the Philippine Republic has increased production about 40% in the same period. Data of the United States Department of Agriculture on production of copra in the Philippines for the years 1947-53 are given in Table II (6-8). These estimates are approximately the same as those reported by the Intelligence Branch of the Commonwealth Economic Committee (Table I).

TABLE II
COPRA, COCONUT OIL, AND DESICCATED COCONUT PRODUCTION IN THE PHILIPPINE
REPUBLIC (COPRA EQUIVALENT)^{a-c}

Copra equivalent	Average 1935-39	Average 1947-51	1950	1951	1952 ^d	1953 ^d
short tons						
Exports						
Copra	335,800	806,946	774,729	851,240	729,966	663,339
Coconut oil as copra ^e	287,500	98,019	123,364	137,097	141,268	104,158
Desiccated coconut as copra ^f		68,649	95,112	56,785	50,615	63,582
Total as copra	623,300	973,614	993,205	1,045,122	921,849	831,079
Domestic utilization						
Coconut oil as copra ^e		99,719	117,204	115,556	124,444	138,667
Total production as copra ^g		1,073,333	1,110,409	1,160,678	1,046,293	969,746

^a U. S. Dept. Agr. Foreign Agr. Rept. 11, 43 (1946).

^b U. S. Dept. Agr. Foreign Agr. Service Circ. FFO 7-53, March 23, 1953.

^c U. S. Dept. Agr. Foreign Agr. Service Circ. FFO 2-54, April 1, 1954.

^d Preliminary.

^e Computed at an extraction rate of 63% of the oil.

^f Computed as 83% of copra equivalent.

^g Excluding coconut utilized in the making of home-made oil and native culinary preparations.

Not included in these estimates of world production of copra is an unknown quantity of coconuts consumed as fresh nuts or utilized in the preparation of home-made oils and other culinary products. In 1946, it was estimated that, in most areas, at least 20% of the nuts produced were used for domestic consumption (6).

2. World Trade

a. Copra

The exports of copra from the principal exporting countries are given in Table III (4). As one would expect, the Philippine Islands and Indonesia are the largest exporters as they produce almost 60% of the

TABLE III
EXPORTS OF COPRA: LEADING COUNTRIES (1938, 1948-54)^a

Country	1938	1948	1949	1950	1951	1952	1953	1954
1000 short tons								
Asia								
Philippines	377	646	582	769	851	739	665	841 ^b
Indonesia	614	356	427	377	616	403	344	352 ^b
Ceylon	84	62	25	24	21	46	24	52
British Malaya	208	66	100	140	101	68	76	73
British Borneo	15	9	25	40	27	17	17	18
Southwest Pacific								
New Guinea	83	21	41	53	72	69	72	80
Papua	12	9	10	12	12	17	13	13
Fiji	37	24	16	11	17	13	7	4
New Hebrides	12	24	25	24	30	24	25	26
French Oceania	22	22	28	22	28	27	19	24
Gilbert and Ellice Islands	6	4	6	9	4	7	8	7
Solomon Islands	26	3	9	11	16	15	18	21
Western Samoa	12	16	18	16	17	19	12	16
Africa								
Mozambique	43	46	49	47	41	43	43	40
Zanzibar	13	1	9	0	0	3	7	7
Tanganyika	4	0	9	4	0	7	2	3
Other countries	64	53	41	54	73	54	63	65
World total	1632	1362	1420	1613	1926	1571	1415	1642

^a U. K. Commonwealth Economic Committee, "Vegetable Oils and Oilseeds." H. M. Stationery Office, London, 1953, 1954, and 1956.

^b Philippine exports increased to 880 and 1,030 thousand short tons in 1955 and 1956 respectively. Indonesian exports decreased somewhat in the same period. *U. S. Dept. Agr. Foreign Agr. Service Circ.* FFO 8-57 (1957).

world copra. Since the war, the Philippine Islands have become by far the most important single source of copra entering world trade.

The principal importing countries are given in Table IV (4). The leading net importers of copra are the United States, Germany, the Netherlands, and the United Kingdom. A decline in the production of soap due to competition from synthetic detergents, together with the low price of domestic tallow and greases, has resulted in a reduced level of purchases in the United States during recent years. This trend may change as coconut oil finds new industrial outlets, primarily in the manufacture of synthetic detergents (8a).

The report compiled in the Intelligence Branch of the Commonwealth Economic Committee (4) in London gives an excellent analysis of the world trade in copra and coconut oil as do the publications of the Food and Agriculture Organization of the United Nations (5), and the U. S. Department of Agriculture (8a).

TABLE IV
IMPORTS OF COPRA: LEADING COUNTRIES (1938, 1948-54)^a

Country	1938	1948	1949	1950	1951	1952	1953	1954
1000 short tons								
United States	256	448	428	469	449	326	324	336
Germany	306	24	72	78	128	167	171	221
Netherlands	58	158	198	199	315	159	161	195
United Kingdom	128	113	119	127	200	188	103	120
France	158	105	106	57	116	124	91	100
Malaya	132	99	128	143	113	100	87	148
Denmark	83	45	52	50	73	72	56	60
Sweden	44	34	47	45	56	43	41	54
Norway	49	22	25	31	34	30	40	43
Australia	21	27	32	37	24	36	37	29
Japan	10	25	18	41	44	29	32	45
Venezuela	0	11	7	24	15	18	30	44
Belgium	26	15	12	26	85	95	29	38
Switzerland	13	32	28	43	30	26	29	30
Canada	0	37	28	25	29	35	12	24
Italy	32	24	25	18	36	24	7	16
India	{ 48	6	7	16	11	22	22	49
Pakistan		17	9	10	10	25	3	8
Others	215	120	125	80	109	123	75	87
Total	1579	1362	1466	1519	1877	1642	1350	1647

^a U. K. Commonwealth Economic Committee, "Vegetable Oils and Oilseeds." II. M. Stationery Office, London, 1953, 1954, and 1956.

b. Coconut Oil

Exports of coconut oil in 1953 totaled 346,300 short tons, and in 1954, 361,200 short tons (4). Since World War II, Ceylon has replaced the Philippines as the leading exporter of coconut oil. Ceylon, the Philippines, and British Malaya are the major exporting countries in the East. In 1954 these three countries accounted for 66% of the total world exports. The Netherlands is becoming an important exporter of coconut oil as well as a processor of copra. The Netherlands exported 45,900 short tons, 13% of total world exports, in 1953.

The United States has always been a large importer of coconut oil. Since 1951, Germany has exceeded the United States in imports and has become the leading importer. These two countries imported 41% of the total world imports in 1953. Other leading importers are the United Kingdom, Italy, and India.

3. Production of Coconut Oil Meal

Referring to Table I, the estimated production of copra, and Table III, the exports of copra, one can see that through the years more than 50% of the copra produced has been exported. In 1938, 57% of the copra production was exported; in 1952 this figure was 52%; in 1953, 47%; and in 1954, 52%. Since much of the copra that remains in the country where it is produced is not processed, it is apparent that a large percentage of the coconut oil meal for feeding is processed in countries other than where the coconuts are grown.

Table V gives an estimate of the coconut oil meal produced in the major processing countries for which data are available. Except for the United States, the Philippines, and India, these data are calculated from production figures and import and export data. It was assumed that all copra produced or imported, less the amount exported, was processed in the country. The oil extraction yield was assumed to be 63%, with the knowledge that this may be too high for some areas. Coconut oil meal production for the Philippine Republic was calculated from oil production data (8). Data for India were calculated from oil production figures based on a 50% oil-extraction yield (9). Figures on coconut oil meal production in the United States for the five years prior to October 1, 1954, given in Table V, are United States Department of Agriculture data (10).

Among the copra-producing countries, the Philippine Republic, Indonesia, India, Ceylon, British Malaya, and Fiji are the only ones that process substantial quantities of copra. The oil produced in Indonesia and India is practically all consumed in those countries. The other major copra-processing countries are importers of copra.

a. Areas of Processing

(1) *Indonesia*. In 1939 there were 69 mills operating in the then Netherlands Indies, which produced 201,300 tons of coconut oil (6). In addition to coconut oil produced in the oil mills, large quantities are produced in villages for local consumption. All coconut oil produced is consumed domestically (2). If copra export (Table III) is balanced against copra production (Table I), it is apparent that approximately 336,000 tons of copra were available for processing in 1952, and 463,000 tons in 1953. This makes Indonesia the leading copra-processing country.

(2) *Philippine Republic*. Referring to Table II, it is evident that a larger percentage of the copra was processed within the Philippine Republic before World War II. Calculations from the data of the United States Department of Agriculture show that an average of about 287,400 short tons of copra were processed annually in the Philippines for 1935-39, yielding about 106,300 tons of coconut oil meal annually. The average annual crush of copra for the

TABLE V
ESTIMATED COCONUT OIL MEAL PRODUCED^a

Country	1952	1953			
1000 short tons					
Copra-producing countries					
Indonesia	124.3	171.3			
India ^b	112 (1952-53)	118 (1953-54)			
Philippine Republic ^c	98.3	89.8			
Ceylon	90.7	88.4			
British Malaya	75.5	67.0			
Fiji	11.8	11.1			
Copra-importing countries					
Germany	61.8	63.3			
Netherlands	58.8	59.6			
United Kingdom	69.6	38.1			
France	45.9	33.7			
Denmark	26.6	20.7			
Sweden	15.9	15.2			
Norway	11.1	14.8			
Australia	13.3	13.7			
Japan	10.7	11.8			
Venezuela	6.7	11.1			
Belgium	35.2	10.7			
Switzerland	9.6	10.7			
Canada	13.0	4.4			
Italy	8.9	2.6			
Year beginning October 1					
	1949	1950	1951	1952	1953
United States ^d	154	149	122	116	116

^a Based on copra production and imports less exports; computed on yield of 63% oil on extraction.

^b Calculated from coconut oil production data; computed on yield of 50% oil on extraction; *U. S. Dept. Agr. Foreign Agr. Service Circ. FFO 9-55, May 5, 1955.*

^c Based on exports of oil and oil used domestically; computed on yield of 63% oil on extraction; *U. S. Dept. Agr. Foreign Agr. Service Circ. FFO 2-54, April 1, 1954.*

^d Data of United States Department of Agriculture; *U. S. Dept. Agr. Agr. Marketing Service FDS-149, Jan. 4, 1955.*

years 1951-53 was 253,730 short tons, yielding 93,900 tons of coconut oil meal (calculated from Table II). Much of the crushing capacity in the Philippines was destroyed during the war in 1942-44. These production data indicate that about 88% of the processing facilities had been repaired or replaced by 1953.

(3) *Ceylon*. In 1951 it was estimated that the milling facilities in Ceylon were large enough to process all the copra produced, but a lack of storage facilities made it impossible to mill all the copra produced during the rush season or to satisfy a sudden large demand for oil (11). Ceylon is now exporting only about 10% of its copra production, whereas the coconut oil ex-

ports represent about 70% of the copra production. If it is assumed that some oil is consumed within the country, one can see that Ceylon processing plants must crush between 185,000 and 200,000 tons of copra per year.

(4) *British Malaya*. In 1950 there were 9 copra processing mills operating in Singapore, and 69 mills in the Federation (11). Mills in Malaya produced 101,875 short tons of coconut oil in 1949, and 101,326 short tons in 1950. Malaya processes about the same tonnage of copra as is produced. Much, if not all, of the large export of copra from Malaya is balanced by the large importation of copra from Indonesia for reshipment.

(5) *Fiji*. The establishment of a crushing industry in Fiji was brought about by a long-term contract for oil with the United Kingdom (4). In 1953, about 80% of the copra production of Fiji was exported as coconut oil. Most of the remainder was exported as copra. The crushing capacity in Fiji apparently totals at least 29,000 short tons per year.

(6) *India*. India has a deficit position in regard to coconut products, in spite of the fact that it is among the leading world producers (9, 11). It was estimated that 211,800 short tons of copra were crushed in India in 1950 (11). Approximately 40% of the coconut production in India is converted to copra and crushed for oil (9). This amount would correspond to about 230,000 tons of copra processed for oil in 1953-54 and 1954-55 (9). All the coconut oil and presumably all the coconut oil meal are consumed within the country.

(7) *Other Copra-Producing Countries*. Other copra-producing countries either importing copra or exporting small amounts of coconut oil, and therefore having processing facilities, are Tanganyika, Zanzibar (11a), Pakistan, and China (Hong Kong) (4). Oil mills also started operations in New Guinea in 1953.

(8) *United States*. The United States processes more copra than any other copra importing country. In recent years all the copra has come from the Philippines (2). Processing plants are located on the Pacific Coast in California, and on the Atlantic Coast.

b. Coconut Oil Meal Trading

Practically no data are available on world trade of coconut oil meal. It is assumed that little coconut meal enters the export trade for it is used primarily for animal feed and fertilizer, and its low value does not justify the costs of shipping in most cases.

Limited data are available on the amount of coconut oil meal imported into the United States (10). The coconut oil meal imports for the five years prior to October 1, 1954, reached a peak in 1951-52 of 104,000 short tons. In 1949-50 this figure was 57,000, and in 1953-54 it was 80,000 short tons. A large part, if not all, of this tonnage is imported from the Philippine Republic.

In general, the European countries that import copra rather than coconut oil do so because they make extensive use of the coconut oil meal for livestock feed. Practically all of the coconut oil meal is fed in the country where it is produced.

In Ceylon, poonac, a by-product of the milling of copra, is used

as fertilizer and animal food (11). One of the purposes of the development of the crushing industry in Ceylon was to have the coconut oil meal available in addition to exporting the more valuable oil.

c. Coconut Oil

Coconut oil is widely used in both the industrial and edible fields. Because of its high lauric acid content and quick lathering properties, the largest application has been in the manufacturing of soap. Coconut oil fatty acids combined with sodium make a hard soap which is also fairly soluble in water. In recent years coconut oil and coconut fatty acids also have been used in the manufacture of synthetic detergents (12). The high melting point of the oil, about 76°F., has made it an excellent oil for confectionery, margarine, and bakery products. It also has been used as a plasticizer in various products and was a necessary ingredient in the manufacture of synthetic rubber during World War II.

Coconut oil is made up primarily of glycerides of saturated fatty acids (13). It has an iodine value of 7 to 10 and a saponification value range of 251 to 264. Lauric acid is present in the glyceride in the largest amounts, 44 to 52% of the total. An excellent review and more detailed description of coconut oil and its uses can be found in a book by Eckey (13).

d. Miscellaneous By-Products

(1) *Coir*. In both India and Ceylon there is an important native industry in the making of rope, or coir, from the fiber of the husks of coconuts. By a process known as "retting," the fiber is softened in water and then spun into yarn.

(2) "*Toddy*," "*Arrack*," and "*Jaggery*." In India and Ceylon these preparations are made from the sap taken by tapping the flower bud. If the juice is permitted to ferment, toddy is obtained. If it is distilled, the product is known as arrack. Jaggery is the sugar made by boiling down the fresh toddy.

(3) *Desiccated coconut meat*. Known more commonly in the United States as shredded coconut, production of desiccated coconut meat is an important industry of Ceylon, the United States, and the Philippines. It is used by the confectionery and bakery trades.

4. Prices of Copra, Coconut Oil and Coconut Oil Meal

Prices of copra and coconut oil at four points in the world and of coconut oil meal in the United States are given in Table VI (5, 14).

TABLE VI
COPRA, COCONUT OIL, AND COCONUT OIL MEAL WHOLESALe PRICES
IN SELECTED COUNTRIES

Year	Copra ^a				Coconut oil ^a				Coconut oil meal ^b
	India	Malaya	Philip-pines	United States	Ceylon	India	Philip-pines	United States	United States
dollars per 100 lb.									
1934-38		2.00	1.91	2.50	2.86	5.09	3.91	7.00	1.24
1947		7.37	7.96	10.00	13.00	20.32	18.18	20.70	4.27
1949	16.29	9.96	7.05	8.80	12.09	24.32	14.09	17.40	3.20
1951	17.02	10.83	8.19	10.40	15.18	23.00	15.91	18.50	4.50
1952	12.19	7.14	5.64	7.50	9.14	19.45	10.45	13.60	4.13
1953	11.88	9.24	8.28	10.60	12.00	18.14	15.45	19.00	3.38
1954	11.01	8.01	7.01	8.90	10.55	15.86	12.95	16.20	—

^a According to the *Food and Agr. Organization U. N. Monthly Bull. Agr. Econ. and Stat.* 4 (10), 55 (1955).
^b Wholesale price, bagged, Los Angeles; *U. S. Dept. Agr. Agr. Marketing Service Stat. Bull.* 159, 72, 79 (1955).

These prices show the increase that has taken place since World War II and the relative value of the products. They also emphasize what was pointed out previously, that copra is valued largely for its oil content. The relative value of copra and oil in the United States and Philippines is quite similar. The difference is due primarily to the freight cost for shipment to the United States.

III. STRUCTURE AND COMPOSITION

The literature on structure and composition of copra has been reviewed by several writers through the years. The reader is referred to the several books for more detailed information than is supplied in this review (15-17).

1. Coconut Culture

The coconut palm is found growing in the tropical belt which extends 20° to 25° north and south of the Equator. Opinions differ as to where the tree originated. Some authorities maintain that it is indigenous only to tropical America and that the nuts were carried by water to other parts of the world. The more general belief, however, is that the coconut originally was native to some place on the Indian Ocean and was carried eastward by man (17).

The major portion of the world crop probably is grown on trees which receive no systematic cultivation, trees planted largely by natives in small groves. They remain untended from that time until they start to bear nuts, in five to fifteen years, depending on growing conditions. They have a long bearing life of fifty to eighty years.

The tree is a monocotyledon and during its life forms only one vegetative bud from which the leaves develop. As many as 6 nuts will develop with each flowering branch or leaf. The annual crop of any tree must be harvested at various times throughout the year, since the nuts reach maturity at different times, after about a year from flowering.

Under favorable conditions, the trees produce 250 or more nuts per year (13). In 1928 the over-all average world production was estimated to be about 25 nuts per tree per year (16). In the Philippines, for 1953 it was estimated that there were over 2.6 million acres of coconut trees, with 164 million trees that produced 3.7 billion nuts for a total of 969,746 short tons of copra. This would be an average of 62 trees per acre, 23 nuts per tree, and 731 pounds of copra per acre, or 461 pounds of oil and 270 pounds of coconut oil meal per acre (8). In 1952, the yield was estimated to be 857 pounds of copra per acre (7).

In some areas the coconuts are harvested by cutting or picking them from the trees, often in an under-ripe condition; in other areas, they are permitted to ripen and drop to the ground. Difference of opinion exists as to which is the better method.

2. Environmental Requirements

The coconut palm, like other tropical palms, thrives in areas having a mean annual temperature of 72°F. or higher. It can endure temporary decreases in temperature to as low as 40° to 50°F.

The tree does not store much moisture and consequently is especially adaptable to areas where there are no periods of drought. It should have at least 40 inches of rainfall evenly distributed throughout the year. In many of the best-known coconut-growing countries, the average rainfall is 70 to 80 inches. A circulating ground-water supply is another important requirement. Usually, coconut palms are planted on coastal areas where water from adjacent coastal ranges may percolate through the soil.

The tree is essentially a lowland plant. As a rule, it flourishes only at low levels, particularly along the coast, but is grown commercially at 1500 feet or higher if other conditions are especially favorable. It has no taproot and, therefore, is not deeply implanted in the soil. Hard.

stiff soils are not so satisfactory because the roots are too weak to penetrate deeply. Although the coconut palm will grow satisfactorily in practically any well-drained soil, deep alluvial plains are best. Sandy soils near the coast and land of volcanic origin are also favorable.

3. Composition of Raw Copra

Much of the information on the structure and composition of raw copra has been taken from the reviews by Eckey (13) and Winton and Winton (18).

Copra is obtained from the fruit portion of the coconut, which is somewhat larger than a man's head and has a blunt-pointed tip. The fruiting branches grow from the axils of the leaves. Flowering occurs throughout the year. The oil accumulates only near the end of the growing period.

The nut which contains the copra is covered by a thick, tough epicarp layer and a mass of fibrous tissues forming the mesocarp. The epicarp and mesocarp together make up what is referred to as the husk. Below the husk is the hard, brown, woody shell, or endocarp, which is several millimeters thick. Within the endocarp are the thin, soft spermoderm and the endosperm which make up the portion that is referred to as copra when broken out and dried. The cavity in the nut is filled with "coconut milk," which may be considered as part of the endosperm and which completely fills the cavity when the fruit is young. "Coconut milk" contains up to 7% sugar, less than 1% protein, and less than 3% fat, together with salts, gums, and other constituents (13). It is mostly wasted in the production of copra. The meats from the freshly opened nuts contain about 50% moisture. They will mold and deteriorate unless the moisture content is reduced promptly after the nuts are opened (19).

Dried copra usually contains 63 to 68% oil and 4 to 7% moisture (13). Some kiln-dried samples containing as much as 74% oil have been reported (13). Dried copra contains about 6 to 9% protein. Because of the many and varied crude methods for harvesting and preparing dried copra, it varies considerably in composition. Copra is graded by buyers either by visual inspection or chemical analysis.

IV. PROCESSING

Coconut oil occasionally is expressed from fresh copra at its native habitat. The usual practice, however, is to extract the oil from dried copra at local oil mills or to export it to oil mills in other countries. The

methods of drying, shipping, and extracting the oil may differ in different areas or within an area. Many of the procedures are crude and determine, to a large extent, the quality of the oil and meal produced.

1. Harvesting and Drying

The coconuts are harvested either by cutting or pinching from the trees or by permitting them to ripen and drop to the ground. After they are collected by hand, the copra meat must be removed from the nut and dried. Proper drying of the copra is necessary for the preservation of the quality of the oil. This operation commonly takes place in the coconut grove near the site of copra production.

The most common method of preparing the copra is to separate the husks from the nut by hand labor and then to split the nuts in half. After the milk is drained off, the halves are dried sufficiently to loosen the meat from the shell. This preliminary drying may be done in the sun or by artificial heat. The separated meats are then dried further. In some areas, the complete drying of the copra in the sun is not practical, and a great variety of methods have been devised. The one in greatest use is the so-called "smoke drying" in which the copra is placed on simple grids and heated by hot gases from fires made with coconut shells. This usually takes place near the trees where the nuts have been harvested. The smoke also has a preservative effect, inhibiting the growth of molds and other microorganisms. Most of the copra produced in the Philippines, which is the principal source of coconut oil used in the United States, is smoke-dried. In a few places modern furnaces for drying copra are in operation, but they can be afforded only by large estates or by groups of natives operating them on a cooperative basis.

In 1906, Marot was issued patents for subjecting fresh copra to treatment with sulfur dioxide in a closed vessel and then drying the product in the air (20, 21). Copra so treated has been shown to have superior keeping qualities for as long as three years (22). Oil expressed from this copra was colorless and free from rancidity (23). Sulfur burners which give a combination of drying and sulfur treatment also have given satisfactory results (23-25).

It is difficult to introduce improved methods for drying copra produced by natives. The drying takes place at so many different points on an island, and by so many different people, that the teaching of modern methods is a slow process. Cost of modern equipment also eliminates its use by many of the smaller operators.

Proper handling of copra in storage and in shipment, as well as in drying, has an important effect on the final quality of the oil produced.

The dried copra is transported by the many different forms of local transportation either to a local oil mill or to a collection point for export where it may be stored for several weeks or months before shipping space is available for exportation. At some of the larger collection points, equipment is also available for additional drying to a moisture content of 4 to 7%.

2. Oil Extraction Techniques

a. Processing Methods

Several primitive methods have been employed in the past and to a small extent at present to supply oil for local consumption. The simplest method is to boil the crushed fresh meats in water and skim off the oil that floats to the top. This method, naturally, gives a very poor yield of oil.

Another type of mill that found great use in India and Ceylon in the past was the mortar-and-pestle type called "chekku." The mill was usually made of a section of tree trunk of hard wood, but sometimes it was made of stone or iron. The interior was shaped in the form of a large mortar. A wooden pestle was attached to a lever in such a way that it could be rotated by bullocks walking around the mortar. The oil cake, or residue, was forced against the sides of the mortar while the oil accumulated in the center. The oil then was soaked into cloths, or otherwise removed from the mortar. These mills were operated with dry copra and could handle about 42 pounds at one charge (13). Coconut oil meal produced by crushing copra in a chekku in Ceylon (about 1940) contained 15% residual oil compared with 8% oil in coconut oil meal produced with more "modern machinery" (26).

Most of the commercial supplies of oil in recent years have been obtained from copra processed in more modern mills, such as those built since World War II. Several types of equipment are used, including all those generally employed for milling oilseeds.

Because of the high oil content of copra, a two-stage process is sometimes adopted. The application of moderate pressure, usually in a screw-press extractor, removes the bulk of the oil; and in the second stage, the residual oil is reduced to a low value by being pressed at high pressure or by being extracted with solvent. Hydraulic box presses and cage presses, continuous mechanical screw presses, and combinations of these with each other are in general use. In preparation for pressing in single-stage screw presses, as is usually done in the United States, the copra is ground in a hammer mill or attrition mill, after which it may

or may not be rolled. Then it is heated to 200° to 220°F. and dried to a moisture content of less than 2½% before entering the press. With modern presses, the residual oil in the press cake can be reduced to less than 6%.

b. Newer Methods of Processing

In recent years there has been some interest in extracting coconut oil with solvent. The greatest use of solvent extraction has been in Germany. At this writing, one copra-processing plant in California is of the prepress, solvent-extraction type. The copra is normally prepressed with a mechanical screw press, and the residual oil reduced to about 1% by a second-stage extraction with solvent. Solvent extraction permits a higher degree of oil extraction than any other method but involves a large capital investment.

In the United States, solvent extraction of copra has not gained favor particularly because of the poor acceptance of the copra meal of low residual oil content for livestock feeding. The residual oil of copra meal is regarded quite highly by many dairy men, since it appears to have a beneficial effect on butterfat percentage that is not observed with other fats. (See also Chapter 24 for a similar statement about palm kernel meal.)

c. Effects of Processing Methods on Meal Quality

Much of the coconut oil meal produced by the older methods of processing was of poor quality for feeding. Some have attributed the difficulty to the high content of oil (26). Most likely the problems arising from feeding coconut oil meal high in oil content are caused by rancidity of the residual oil (27). Fresh coconut meats have been fed to pigs (28) and growing chicks (29) with good results.

The effect of processing methods on protein quality has been studied with conflicting results. Jones *et al.* (30), in 1923, noted that solvent-extracted and screw-pressed meals gave the same results in protein quality studies with rats, indicating that they are of equal nutritive value. Later work by Mitchell *et al.* (31, 32) showed that coconut oil meal produced by a solvent-extraction procedure carried out at a temperature that never exceeded 70°F. had a higher biological value (71% versus 58%) than a product that "had been prepared by the usual drastic methods." Although this comparison was done at two different times, the procedure for determining the biological value with rats was the same; and the work indicates that there can be a difference in protein quality as a result of differences in processing.

Recent work in the Philippines by Loosli *et al.* (33) indicated that the protein, organic matter, fiber, and nitrogen-free extract of fresh coconut meat was more digestible than those of coconut oil meal. It appears from these data that the screw-press method of processing lowers the digestibility by swine of coconut oil meals.

With the exception of meal produced by solvent extraction, which was discussed in the previous section, meal quality for cattle does not appear to be adversely affected by any of the present processing methods. Copra meal is used primarily for dairy feeding, and there is no evidence that any factor other than the residual oil content will affect its feeding value.

3. Effect of Storage on Quality of Product

Copra is often stored for several weeks or months before milling, either in the country where produced or in the importing country. If it is not dried properly immediately after harvest, the action of bacteria and mold will be quite harmful. Considerable damage also occurs in shipment. The principal change that takes place is an increase in the free fatty acid content of the oil. Storage at 6% moisture content or lower is usually considered best for inhibition of molds and bacteria.

Trouble has been experienced with coconut oil meal which had become rancid in warm weather. In 1901, it was noted in California (27) that the one objection to coconut oil meal was its lack of keeping qualities. It would become rancid when stored for several weeks, in which condition it was not relished by livestock; and if fed to dairy cows, it would sometimes produce an off-flavor in the milk. Rancid coconut oil also has produced symptoms of vitamin E deficiency when fed to dairy calves (34, 35). Since coconut oil is considered to be very stable (13) it seems unusual that the residual oil in coconut oil meal should become rancid. The poor keeping qualities that have been observed with copra meal in the past probably were related to the method of processing, moisture content, and storage conditions. High moisture in raw copra is known to favor the development of mold and bacteria and a reduction in oil quality (19). Coconut oil meal, as produced currently, usually has a sufficiently low moisture content, and the oil content is lower because of improved oil extraction, so that it keeps satisfactorily in storage.

V. PROPERTIES OF OIL MEAL

Since the early days of plantation production in Ceylon, the poonac, or extracted copra residue, has been used for cattle feed (16). Coconut oil meal makes an excellent feed for livestock, particularly cattle, because of its protein content, the characteristics of its residual oil, and its palatability. Coconut oil meal also has a high capacity for absorbing molasses and is sometimes used for this purpose in dairy rations. It also has been included to a lesser extent in rations for other livestock.

1. Chemical Composition of Meal

a. General Composition

The general composition of coconut oil meals is given in Table VII (33, 36–38). Its protein content is normally guaranteed at 20% minimum, which makes it a medium-protein feed ingredient. The protein digestion coefficient is 85%, which is close to that of hydraulic- and

TABLE VII
PROXIMATE COMPOSITION OF COCONUT OIL MEAL

Constituent	Processing method				
	Hydraulic and screw press ^a	Screw press ^b	Solvent extraction ^a	Undefined method ^{c,d}	Screw press ^e
	%	%	%	%	%
Dry matter	93.2	93.0	91.1	91.5	91.7
Crude protein ^f	21.3	21.5	21.4	23.1	20.8
Crude fat	6.7	6.6	2.4	11.5	7.1
Crude fiber	10.7	11.3	13.3	11.3	9.9
Nitrogen-free extract	48.3	47.1	47.4	39.5	49.0
Ash	6.2	6.4	6.6	6.1	4.9
Total digestible nutrients	77.7	—	68.6	—	69.9
Number of analyses	22	7	7	—	—

^a F. B. Morrison, "Feeds and Feeding," 21st ed., pp. 573, 669, 1116. Morrison, New York, 1950 (c 1948).
^b W. E. Sewell, Procter and Gamble Co., unpublished data, 1948.
^c The method is not defined but is presumably a crude process as judged by the fat content.
^d A. Khan, *Oils & Oilseeds J. (India)* **4** (4), 17 (1951).
^e J. K. Loosli, J. O. Peña, L. A. Ynalvez, and V. Villegas, *Philippine Agr.* **38**, 191 (1955).
^f Calculated as nitrogen \times 6.25.

screw-pressed soybean oil meal, but is higher than corn. The total digestible nutrient content of coconut oil meal is higher than that of most vegetable oil meals but is about 3% lower than corn.

b. Amino Acids

Results of microbiological assays for the essential amino acid content of the protein in coconut meal are given in Table VIII. The analyses by Lyman *et al.* (39, 40) and by Carroll (41) are for coconut oil meals processed in the United States from Philippine copra. The origin of the coconut oil meal analyzed by Williams (42) is not reported.

Examination of these data indicates that lysine most likely would be the first limiting amino acid in coconut oil meal for feeding to non-

TABLE VIII
ESSENTIAL AMINO ACID CONTENT OF COCONUT OIL MEAL

Amino acid	Grams per 16 g. of nitrogen			
	a	b	c	d
Arginine	11.2	10.4	10.3	10.9
Histidine	1.7	1.8	1.6	1.6
Isoleucine	4.0	3.4	3.9	5.0
Leucine	4.9	5.4	6.7	7.0
Lysine	2.5	2.2	2.3	2.8
Methionine	1.5	1.5	1.8	1.4
Phenylalanine	4.3	5.1	4.1	3.9
Threonine	3.7	3.0	3.4	3.0
Tryptophan	0.88	0.99	0.9	0.86
Valine	5.5	5.1	6.1	5.0
Total protein, %	21.5	—	20.7	20.9
Number of samples	2	3	3	2

^a C. M. Lyman, K. A. Kuiken, and F. Hale, *Texas Agr. Expt. Sta.*, unpublished data (1946).

^b C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).

^c R. W. Carroll, Quaker Oats Co., unpublished data (1955).

^d H. H. Williams, *Cornell Univ. Agr. Expt. Sta. Mem.* **337**, 31 pp. (1955).

ruminants. Histidine content is lower in coconut oil meal than in other high-quality protein sources (42) but will probably be adequate in most rations. Coconut oil meal protein is high in its content of arginine.

c. Fat

Mechanically-pressed coconut oil meal contains about 6.6% fat compared to about 2.4% fat in solvent-extracted meal (36, 37). Feeders in the United States have been hesitant to buy solvent-extracted coconut oil meal because of its lower fat content. Feeding tests have shown that coconut oil meal will increase the fat test of milk (36), and it is generally believed that this increase is due to the character of the fat in coconut oil meal. Coconut oil meal and coconut oil also have been shown to contribute to a desirable firm texture of fat on hogs and to a lowered iodine value of the pork fat (43-45).

d. Vitamins

Although coconut oil meal does not have an abnormally high content of any of the vitamins, it will contribute significant amounts of the B-complex vitamins to a ration in which it is included. Analyses for

some of the vitamins are given in Table IX (36, 37). As with similar vegetable proteins, one would not expect coconut oil meal to contain vitamin B₁₂, and no report has indicated its presence.

TABLE IX
VITAMIN COMPOSITION OF COCONUT OIL MEAL

Vitamin	Milligrams per pound ^a	Milligrams per 100 g. of meal ^b
Tocopherols		2.10
Thiamine	0.3	0.06
Riboflavin	1.2	0.27
Niacin	13.3	2.93
Pantothenic acid	3.2	0.71
Pyridoxine		0.25
Folic acid		0.014
Biotin		0.023
Inositol		270.00
Choline		110.00
<i>p</i> -Aminobenzoic acid		0.21

^a F. B. Morrison, "Feeds and Feeding," 21st ed., pp. 573, 669, 1116. Morrison, New York, 1950 (c 1948).

^b W. E. Sewell, Procter and Gamble Co., unpublished data, 1948.

e. Minerals

Coconut oil meal, like other vegetable oil meals, is relatively high in phosphorus content. It is also higher in calcium than are the farm grains. Mineral analyses of coconut oil meal are given in Table X (36-38).

f. Miscellaneous Constituents

Nothing detrimental to animal growth or production has ever been reported in coconut oil meal. The use of coconut oil meal in poultry and swine feeds, however, is limited because of the relatively high ratio of fiber to protein in the meal (Table VIII).

2. Use in Livestock Feeding

a. Dairy Cattle

The chemical composition of coconut oil meal would indicate that it would find greatest application in cattle feeds. Coconut oil meal is fed mainly to dairy cows in the United States and is an excellent dairy feed. Feeding of coconut meal has oftentimes caused a slight increase

TABLE X
MINERAL CONTENT OF COCONUT OIL MEAL

Mineral	a	b	c
	%	%	%
Calcium	0.21	0.18	0.21
Phosphorus	0.64	0.69	1.8 (P ₂ O ₅)
Potassium	1.95	1.12	1.6 (potash)
Sodium		0.04	0.6 (soda)
Chloride		1.39	
Magnesium		0.26	
Sand			2.3

	parts per million
Iron	1954.0
Copper	18.7
Cobalt	2.3
Fluorine	3.1
Iodine	None found
Manganese	40.0

^a F. B. Morrison, "Feeds and Feeding," 21st ed., pp. 573, 669, 1116. Morrison, New York, 1950 (c 1948).
^b W. E. Sewell, Procter and Gamble Co., unpublished data, 1948.
^c A. Khan, *Oils & Oilseeds J. (India)* 4 (4), 17 (1951).

in fat content of the milk (46–57). Although these differences in fat content have been small, in some areas coconut oil meal is considered a necessary ingredient in dairy feeds. None of these studies has shown any particular advantage in total milk production from use of coconut oil meal as compared with other supplements. According to Warner *et al.* (56), Hansen (58) reviewed the earlier literature in Europe and reported that as early as 1864 studies in England showed increases in the fat test of the milk after the feeding of coconut oil meal and palm kernel meal. (See also Chapter 24.) In an Ohio trial (59, 60) the fat test was not increased by feeding coconut oil meal. In this test, 3% more milk was produced on a ration containing 30% coconut oil meal.

Coconut oil meal has been compared with several different protein supplements in this respect. In 1918, Ewing and Spence (47) reported that coconut oil meal in a ration for dairy cows produced an increase of 0.13% in fat test when compared with cottonseed meal in an otherwise similar ration. McCandlish and Weaver (50) compared coconut oil meal and linseed meal for milking cows and observed an increase of 0.42 percentage unit in fat test of the milk when the coconut oil meal was fed. In the aforementioned Ohio trial (59), coconut oil meal was compared with a mixture of one-quarter linseed meal, one-quarter cottonseed meal and one-half grain; the coconut oil meal produced

slightly greater milk production, with no increase in fat test. Lindsey (46) observed an increase of 0.40% fat in the milk when coconut oil meal was compared with corn gluten feed.

The only recent studies with coconut oil meal for milking cows are those reported by Cornell workers (56, 57). In two tests conducted in two consecutive years, 40% coconut oil meal was compared with a ration containing 40% corn gluten feed and another ration with 40% corn distillers' dried grains. The protein contents of the grain mixtures were equalized at approximately 18% by varying the relationship of corn and soybean oil meal in the rations. The feed which contained 40% coconut oil meal resulted in a statistically significant increase ($P < 0.01$ first year; $P < 0.05$ second year) in the fat content of the milk. The fat test for the cows fed coconut oil meal was 0.21 percentage unit greater than for cows fed corn distillers' dried grains and 0.19 greater than for cows fed corn gluten feed in the first test; and it was 0.17 percentage unit greater than for cows fed corn distillers' dried grains and 0.16 greater than for cows fed corn gluten feed in the second test. The increase in fat content of the milk was not due to a difference in the fat content of the rations, since there was no correlation between fat level in the rations and butterfat percentage. The rations containing corn distillers' dried grains contained the highest level of ethyl ether extract in both tests.

The coconut oil meal fed in the Cornell experiments (56, 57) would be more representative of the coconut oil meal available today than would the meals fed in the other experimental work with dairy cattle covered in this section. Most of the coconut oil meals contain less residual oil now than they did twenty years ago.

Coconut oil meal fed to milk cows also appears to have an effect on the quality of butter produced. It tends to produce a hard butter of excellent quality and flavor (49, 61). According to Lindsey (46), German observers noted that, when fed in excess of 3 to 4 pounds daily per head, coconut oil meal would make too hard a butter. Whether this would be true of the coconut oil meal of lower fat content available today is not known. Brown *et al.* (62) noted that the feeding of 1 pound of coconut oil per day to a dairy cow decreased slightly the iodine number of the resulting butterfat and also reduced slightly the intensity of the oxidized flavor developed in the presence of added copper.

b. Beef Cattle

Very little, if any, coconut oil meal is fed to beef cattle in the United States. Other vegetable oil meals are usually more economical sources of protein for beef cattle.

In a California experiment (63), coconut oil meal and barley were compared with barley and cottonseed meal as a supplement to alfalfa hay and silage for fattening calves. Gains were slightly better for the cattle on the cottonseed meal ration, but the cattle receiving the coconut oil meal required less feed per 100 pounds of gain. In another experi-

ment, coconut oil meal was used as a substitute for one-half of the barley with good results. The author concluded that coconut oil meal can be used as a substitute for up to one-half the barley for fattening cattle when the price of coconut oil meal is equal to or less than that of barley.

Work in England (64) indicates that coconut cake is a valuable feed for fattening steers kept on grass. A daily ration of 4 pounds of a mixture of three parts coconut cake, three parts cottonseed cake, and two parts linseed cake gave the best results. The authors mentioned that the coconut cake was not very palatable, however. At that time (1916), the coconut cake probably contained high levels of oil which may have become rancid.

c. Sheep

Very little work has been published on the feeding value of coconut oil meal for sheep. In a California experiment (65), coconut oil meal produced satisfactory results when fed as a protein supplement with barley and alfalfa hay for fattening lambs. Likewise, early work at Massachusetts indicated that coconut oil meal would be a satisfactory protein supplement for sheep (66).

d. Swine

Coconut oil meal is too high in fiber content to be fed in large amounts to fattening swine. In many parts of the world, however, it may be economical to feed coconut oil meal to swine. Research has shown that approximately 25% can be included in the ration for fattening hogs. Most of the feeding experiments with swine which have been reported were with rations that were deficient in other nutrients, primarily vitamins and minerals. Compared with animal products, coconut oil meal would be deficient in the B-complex vitamins and the mineral elements calcium and phosphorus.

Average digestible nutrients for coconut oil meal as determined with swine by Loosli *et al.* (33) were: dry matter 91.7%, digestible protein 15.2%, and total digestible nutrients 69.9%. Digestion coefficients reported at the same time were: protein 73%, fat 90%, fiber 50%, and nitrogen-free extract 72%.

The response to additions of coconut oil meal to swine rations in experimental work in the Philippines has been quite variable, depending on the other constituents of the ration. Copra meal alone did not promote growth of pigs but furnished a fairly complete feed when supplemented with green leaves (67). Coconut oil meal added to a corn-rice bran ration for six-month-old pigs increased the gain in weight when levels of coconut oil meal of 30%

or less were used (68). With 40% coconut oil meal in the corn-rice bran ration, the ration was unpalatable, gains decreased, and the pigs were constipated.

When compared with those fed dried shrimps, pigs fed coconut oil meal grew more slowly and required more feed per unit of gain (69). In a more recent experiment, Garcia (70) compared combinations of coconut oil meal and fish meal added to a corn-rice bran ration. With two- to four-month-old pigs, 10% fish meal and not more than 20% coconut oil meal were required for best growth and feed conversion. With six-month-old pigs, a combination of 6% fish meal and 19% coconut oil meal was as satisfactory as 8% fish meal and 17% coconut oil meal.

Early work in Oregon (71) indicated that 25% coconut oil meal and 75% barley gave better results with pigs than barley alone, although it was noted that 50% coconut oil meal in the ration was less palatable. In Iowa work (72), 11% coconut oil meal added to a protein supplement containing tankage, linseed meal, cottonseed meal, peanut meal, alfalfa, and minerals resulted in the same amount of feed required per unit of gain but with a reduced rate of gain (1.27 versus 1.40 pounds per day). In contrast, a Delaware experiment with 139-pound pigs showed that corn and coconut oil meal produced faster gain with a higher degree of finish than corn alone, or a combination of corn and tankage, velvet bean meal, soybean oil meal, or corn gluten feed (73). With 139-pound pigs, however, the requirements for growth are much less critical than for the weanling pig. In 1922, California workers noted that coconut oil meal, when not rancid, could be fed with barley in proportions of one part to three or four parts barley on alfalfa pasture (74). In dry lot, animal protein should be provided (75). Washington Agriculture Experiment station workers found that coconut meal could successfully replace mill run in hog rations (76).

In 1925, Edwards, at Guam, wrote, "Many of the local farmers are beginning to appreciate the value of coconut meal as a feed for livestock, but others still regard the product with suspicion and even with prejudice" (77). Coconut oil meal and tankage produced good gains when fed with corn (77, 78). Coconut oil meal alone, when fed to pigs weighing 84 pounds, produced satisfactory results.

In 1921, Robison at the Ohio Agriculture Experiment Station reported that coconut oil meal was not so efficient as tankage, fish meal, or linseed meal for supplementing corn fed to hogs (79). When using a more complete basal ration, however, supplemented with alfalfa meal and minerals as sources of vitamins, calcium, and phosphorus, Robison showed that coconut oil meal could be used at a 15 to 25% level in the ration to replace corn and other protein supplement and have a higher value than corn (80, 81). When 15% coconut oil meal replaced all the linseed meal in the ration and part of the corn, the average daily gain was slightly greater (1.20 versus 1.45), with the same amount of feed required per unit of gain (81). The coconut oil meal used in the study contained 8% fat and was kept in sacks for more than a year without

becoming rancid or showing evidence of a decrease in palatability when fed to pigs.

In some areas, fresh coconut meats are fed to pigs. In New Zealand (44) the addition of 1 pound per day of copra, and in the Philippines (82) the replacement of one-half or all the corn with fresh grated coconut meats, resulted in satisfactory gains. In the latter experiment, it was noted that the coconut meats were readily eaten.

e. Poultry

Poultry require rations very carefully formulated with respect to content of protein, energy, minerals, and vitamins. Like other vegetable oil meals, coconut oil meal is deficient in vitamins, calcium, and phosphorus for poultry. In addition, the relatively high fiber content of copra meal limits its use in poultry rations. Ewing, in a review (83) written in 1951, stated, "Many feed manufacturers, particularly on the Pacific Coast, have used copra, or coconut oil meal, in poultry mashes with satisfactory results. It is being used by some feed manufacturers at from 2.5 to 5 per cent of the mash formula. Due to its high fiber content, its energy value is low. It is used at a small percentage sometimes in crate fattening mashes, because of its water absorption qualities. Coconut oil meal absorbs molasses up to as high as 50 per cent, and some feed manufacturers use this means of including molasses in their various feed formulas in a convenient form."

In areas where coconut oil meal is produced, it is desirable to use it for economic reasons. Feeding tests have indicated that best results are obtained when it is combined with an animal protein such as fish meal. Work in the Philippines has shown that a combination of 5% coconut oil meal and 20% of either fish meal (84) or shrimp meal (85) was the best combination to feed to growing chicks. Luna (85) noted that the combination of 20% shrimp meal and 5% coconut oil meal was slightly superior to 25% shrimp meal. Higher levels of coconut oil meal were unpalatable, increased the feed required per unit of gain, and increased mortality. Lionag (86) reported that a combination of 10% shrimp meal and 10% coconut oil meal was not so satisfactory for growing chicks as was 20% shrimp meal. Other work also has emphasized the lack of palatability and the limitations in the use of coconut oil meal for chicks (87-89).

Early work with coconut oil meal for laying hens showed that it was unsatisfactory for egg production (90, 91). Later work at Pennsylvania (92) showed that coconut oil meal in combination with dried buttermilk and minerals was approximately equal to meat scraps and buttermilk as the protein source for egg production. The same workers (93) also noted that the coconut meal had no detrimental effect on fertility and hatchability. In 1926, Crucillo (94) in the Philippines noted that coconut oil meal may be used in a supplement for laying hens provided it forms not more than 30% of the ration. In a more recent study over a period of six months, Temperton and Dudley (95)

compared a ration for laying hens that contained 25% bran with the same ration in which the bran was replaced with an equal amount of coconut oil meal. Over a period of six months, egg production, body weight changes, mortality, and returns over feed cost were identical in the two groups.

As noted previously, there are instances when it is desirable to use coconut oil meal for economic reasons. In the Philippines, for example, the goal has been to formulate laying rations that are simple, cheap, easy to mix, practical to use on the farm, and also efficient (96).

Fronza and Campos (96) report that the following ration fed with either rice or corn or both is popular with backyard poultry raisers and farmers in the Philippines:

Ingredient	%
Rice bran	40
Coconut oil meal	30
Ground corn	10
Fish or shrimp meal	20
Shells and green grass—ad libitum	

The following all-mash ration is used for layers at the University of the Philippines with excellent results (96):

Ingredient	%
Ground corn	50
Rice bran	20
Coconut oil meal	10
Fish or shrimp meal	20
Ground shells	2
Green feed—hand-fed every two days	

When given this ration, a flock of Single Comb White Leghorn pullets produced an average of 194 eggs in fifty weeks and required 4.16 pounds (1.89 kg.) of feed to produce a dozen eggs (96). This ration, supplemented with 2% cod liver oil, was also the most satisfactory of ten mixtures that were tested for caged layers.

When as little as 10% coconut oil meal replaced fish meal in a corn-rice bran-fish meal ration for laying ducks, egg production was decreased (97).

f. Horses

Experiments by the United States Department of Agriculture (98) and the Guam Agriculture Experiment Station (99) show that coconut oil meal may be used as a feed for horses with satisfactory results. In the former work, two parts of coconut oil meal and one part of peanut meal were used to replace oats. Although the coconut oil meal and

peanut meal ration was not so palatable, the horses were reported in good condition, and they made greater and cheaper gains than those on oats. In the Guam work, coconut oil meal replaced one-third to one-half the amount of oats usually fed to work animals with satisfactory results.

3. Uses for Human Consumption

Although large amounts of coconut and coconut products are utilized for human consumption in the areas of production, the writer is not aware of any experimental work that has been conducted on the feeding value of coconut oil meal for humans. Kuppuswamy *et al.* (100) reported that coconut cake, when supplementing a South India rice diet, produced marked growth in rats. The reasonably high content of protein of fairly good quality should make it a fairly good supplement to human diets in areas of protein shortage. Likewise, the residual oil in coconut oil meal would have a high value in human diets if it is not permitted to become rancid. Van Veen cites the preparation of products from oil cakes, including coconut meal, by fermentation. These products of higher digestibility and nutritive value than the original cake have been made and used in Indonesia (101). (See also page 218.)

4. Use for Fertilizer

As noted previously, coconut cake, or poonac, is used in Ceylon for fertilizer (11). Recent work in the Philippines showed that coconut oil meal added to Los Banos clay loam improved not only the physical and chemical properties but also the biological properties of the soil which influence its productive capacity (102). The effect of coconut oil meal was decidedly better than that of ammonium sulfate, applied on an equivalent nitrogen basis, as indicated by the growth of lettuce plants. The residual effect of coconut oil meal was also better as indicated by lettuce yield in the second harvest. Andrion (103) showed that coconut oil meal had a beneficial effect on the growth of corn grown on Los Banos clay loam. The increase in yield was more significant with the second planting than with the first. The nitrogen, nitrate, phosphate, potash, and pH of the soil were increased by fertilization with copra meal.

VI. TRENDS

1. Production

The greatest increase in copra production has taken place in the Philippine Republic, in the areas devoted to coconuts in the Provinces of Zamboanga and Davao and in Southern Luzon (8). A considerable

share of the new plantings elsewhere represent the replacement of old trees, worn-out plantations, or areas devastated by the disease kadang-kadang.

Production in Indonesia has increased slowly since World War II but has not reached the prewar level (4). The Copra Foundation, first organized in 1940 as the Copra Fund and reorganized in 1950 as the Copra Foundation, has as its aim "to endeavor to obtain the highest possible benefits from the coconut production for Indonesia and its people in general, and for the community in the coconut growing areas in particular" (4). It has been active in increasing the delivery of copra to the oil mills for export and, in 1952, asked the Agricultural Research Department in Bogor, Java, to train personnel to develop control measures to combat the "artona" worm which reportedly killed 1.5 million coconut trees in West Kalimantan alone in 1952 (104).

Production of copra in Ceylon appears to be at about a constant level. Approximately 15,000 acres of marginal coconut lands in Ceylon are withdrawn annually from production and replanted, but rehabilitation is expensive and slow (2).

The supply of coconut oil for soap-making and other purposes in India has fallen short of the demand, and consequent high prices have encouraged increased production. Moreover, the Indian Central Coconut Committee has inaugurated new planting schemes and has prepared a five-year plan (1956-60) to make India self-sufficient in coconuts for all purposes (4). The Vegetable Oil Development Advisory Board in East Pakistan also has recommended the establishment of a copra industry at various centers in East Bengal (4).

Production in other countries appears to be about constant. Since it takes years to bring a tree into production, one cannot expect widespread changes. Increased yields of copra are possible by improved production techniques; but, in general, this does not appear to be probable in the near future.

What may prove to be a significant development in the promotion of copra growth and processing was the meeting of the First International Coconut Congress held in Manila, August 26-31, 1955, called by the Philippine Coconut Administration, which invited twenty neighboring countries to attend. Questions studied included standardization of quality and prices of copra and other coconut products, creation of a joint propaganda board among coconut-exporting countries for the purpose of developing and promoting world markets, promulgation of a unified research scheme to find new products for the coconut and the solution of existing problems, and discussion of the possibility of turning present copra exports into coconut oil production.

2. Processing Techniques

The trend toward utilization of improved processing techniques in the areas of production has been a slow one, although, since the end of World War II, processing of copra has increased in British Malaya (11) and in Fiji (4). A shift to solvent extraction of copra is expected to proceed very slowly because of the relatively small gain in oil recovery, the large capital investment that is necessary, and the poorer acceptance of solvent-extracted coconut oil meal for feeding to dairy cattle.

3. Market for Products

No significant change in the market for copra products can be foreseen. There should continue to be a need for coconut oil meal for livestock feeding. As mentioned previously, however, the value of the product does not permit extensive world trade. With the encouragement of increased animal production in the areas of copra production, it is natural that coconut oil meal should fit into this program.

In the United States, which has been the leading importer of copra and coconut oil, there is a declining demand for coconut oil because of the decline in the production of soap resulting from the increased use of synthetic detergents. This trend may change as a result of increased use of coconut oil in detergents. In other areas, coconut oil is primarily employed in the manufacture of edible fats, and the increased utilization for this purpose should more than compensate for any reduction in the amounts used in soap.

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CHAPTER 24

PALM KERNEL MEAL

J. G. COLLINGWOOD

I. INTRODUCTION

The history of the use of palm kernels, in Europe at any rate, is not a particularly long one. It is believed that their use in industry dates back to somewhere in the early 'fifties of the nineteenth century. According to Lewkowitsch (1), they were first brought into Marseilles from the French West African colonies as ship's ballast and were jet-tisoned into the sea before reloading of the ship. It was not long, however, before the oil millers of Marseilles realized their value and commenced the production of palm kernel oil to supplement the copra oil already being used in the soap factories there.

Nevertheless, the African oil palm, *Elaeis guineensis* (Jacq), from whose fruit the kernels are obtained, had been harvested probably for centuries before that, references being traced back to 1588 when a British ship first exported it from Nigeria. There is also a reference in 1650 to its use on the Gold Coast (2), where it provided, as it still does, a staple food crop in the form of palm oil, rich in pro-vitamin A. Precise figures are not available, but it is probable that more palm oil is consumed in Nigeria than is exported. The oil from the kernels, however, is hardly used there at all. Export of palm oil on a commercial scale was encouraged by the prohibition of the slave trade, when slave traders found it profitable to change their trade from slaves to palm oil. Great Britain prohibited slave trading in 1807; thereafter, exports of palm oil from West Africa increased rapidly.

The oil palm, like the coconut palm, also provides many other important domestic requirements. Its leaves are used for thatching roofs; the young growth at the top of a tree, known as the cabbage or heart, and the involucreal leaves may be taken from a tree which has been felled and used as a vegetable; the ash from the refuse after the fruit has been stripped from the bunch is rich in potash and is used with oil from the kernels for making soap; and the tree to a small

extent supplements the *Raphia* palm as a source of raw material for quite a large production of palm wine for local consumption. Both the oil and the kernels provide an important source of cash income to the native farmer.

It must be emphasized, however, that the primary commercial crop from the plantations is the oil from the pulp of the fruit (yields of oil per acre are the highest of any known plant—a survey in Sumatra showed yields of oil four to five times as high as that of the oil palm's nearest competitor, the coconut palm), and research, from both the horticultural and processing points of view, is directed mainly toward improving this yield of oil.

As a result, the use of the oil palm as a source of a vegetable protein can be regarded only as incidental. Very little investigational work has been done regarding the nature of the protein, and no significant attempts made to improve or preserve it during processing. The widespread use of palm kernel meal on its own has been retarded by its rather gritty and unpalatable nature, although it forms a useful ingredient in European compound animal feeds, particularly for dairy purposes where it possesses definite advantages. No uses for the meal, other than for animal feeding, have been developed.

II. PRODUCTION AND TRADE

1. Production Conditions

The oil palm, *Elaeis guineensis* (Jacq), is a tree indigenous to the tropics approximately within the latitudes 10°N. and 10°S. It does not need excessive temperatures above 90°F. but requires a minimum rainfall of 55 to 60 inches annually, which must be distributed fairly evenly throughout the year. The highest yields are obtained where annual rainfall is between 80 and 120 inches, and, in the Cameroons, plantation palms thrive with well over 200 inches of rain per year. Growth at altitudes of up to 3000 feet is possible. The tree can thrive in comparatively poor soils, but a good soil will greatly increase the yield of fruit.

It should be made clear that the oil palms are grown in two quite distinct ways, with sometimes quite widely differing purposes in mind. By far the greater quantity are in native palmeries, which are exploited rather than cultivated. Here the oil is required for food for local consumption, and the surplus oil and kernels are exported. The kernels are not used locally, and the revenue from their preparation and sale is the perquisite of the women. Second, there are the plantations in which trees from carefully selected seed are grown for produc-

tion of maximum yield of oil for export, and the oil and kernels are extracted by modern mechanical methods.

2. Areas of Production

The primary areas of production are, in order of importance, West Africa and the Far East, although the palm is also well known throughout tropical Africa, in Central America, and in tropical South America.

TABLE I
WORLD EXPORTS OF PALM KERNELS^a

Country	1909-13	1925-29	1938	1943	1948	1950	1952	1953	1954 (est.)	1955
Thousands of long tons										
Nigeria	174	255	312	331	327	410	374	403	464	443
Sierra Leone	46	64	64	36	66	71	76	69	68	55
Gold Coast	13	6	5	8	7	4	6	7	9	10
Malaya	—	—	9	^b	6	9	11	13	14	12
Gambia	—	1	1	—	1	2	2	2	2	2
Belgian Congo	6	71	87	62	82	83	91	86	70	63
French West Africa	46	67	70	50	62	83	63	84	80	86
Indonesia	—	4	47	^b	11	28	36	41	42	34
French Cameroons	15	34	33	30	30	28	19	21	17	15
French Eq. Africa	—	8	15	8	7	8	8	9	10	8
French Togoland	10	8	9	10	8	13	8	11	9	9
Portuguese Guinea	6	10	13	17	12	13	18	11	12	15
Angola	6	7	7	11	9	11	13	11	10	10
S. Tome and Principe	1	3	2	1	6	7	5	6	4	5
Liberia	^b	^b	12	^b	16	20	23	16	11	10
	323	538	686	564	650	790	753	790	822	777

^a Taken from the following: International Institute of Agriculture; Food and Agriculture Organization of the United Nations, *Monthly Bull. Agr. Econ. and Stat.*; Commonwealth Economic Committee, "Vegetable Oils and Oilseeds," London.

^b Not available.

The original home of *Elaeis guineensis* is not certain, but Cook (2) has suggested that its real home is Brazil. Here it is found in a truly wild state, whereas in Africa, wherever it is found, its presence appears to be associated with man (3a). It is so thoroughly established there, however, that it is always regarded as indigenous to a palm belt in West Africa which stretches from Dakar to the Cameroons. The main producing area for kernels is Nigeria, much smaller quantities coming from the Cameroons, French West Africa, the Belgian Congo, and Sierra Leone. Other areas of production are indicated in Table I. [For a discussion of the oil palm in South America, see Markley (3b, 3c).]

It is estimated that in the Nigerian palm belt land equivalent to some 4 million acres is given to natural palmeries (4). Large commercial plantations and smaller planted areas cover about 40,000 acres with a production of less than 10% of the oil exports and less than 2% of the palm kernel exports. West Africa can, therefore, be regarded as the home of the oil palm.

A second important source of production was started when a few seedlings were transported about a hundred years ago to the Far East

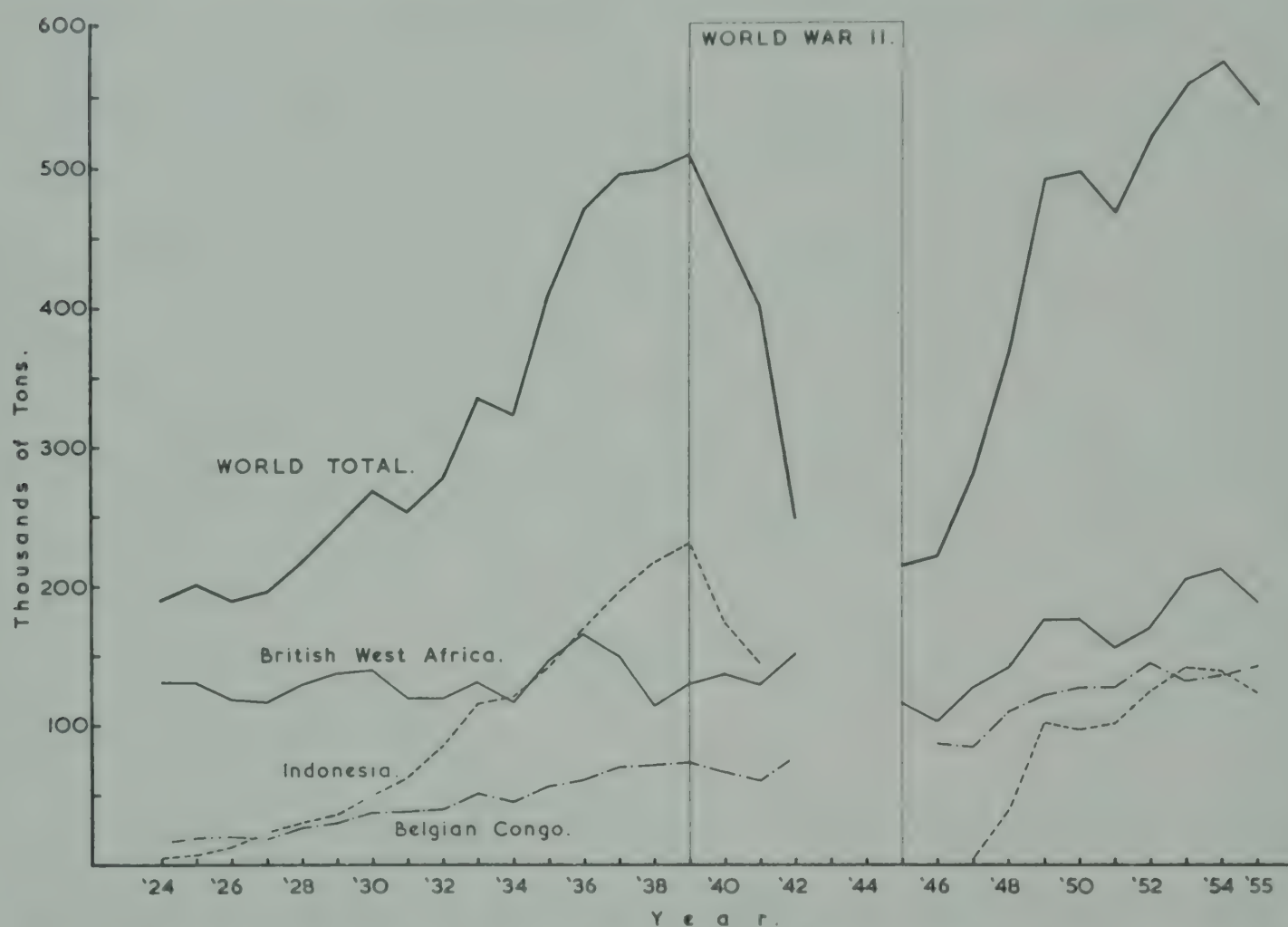


FIG. 1. Annual exports of palm oil, 1924-55. (Reproduced by permission of the Director of the West African Institute for Oil Palm Research.)

where a particular type known as the Deli palm has been extensively and successfully cultivated in plantations.

Prior to World War II, the export of palm oil from Sumatra and Indonesia was approaching two-fifths of the whole world trade, although export of palm kernels was negligible. Considerable losses in the war disrupted this trade, which has not yet returned to its prewar level.

3. Volume of Production

The production of palm kernels is entirely dependent on the production of palm oil, although the ratio of the two from any source may

vary widely, depending on the form of the fruit grown and the economic conditions in the country.

The curves in Figs. 1 and 2 show the sources of world trade in palm oil and kernels and demonstrate the emphasis laid on production of palm oil, almost to the exclusion of kernels, in those areas where scientific methods of cultivation are adopted—that is to say, the areas where the plantations are situated in Indonesia, the Belgian Congo, Nigeria, and Malaya.

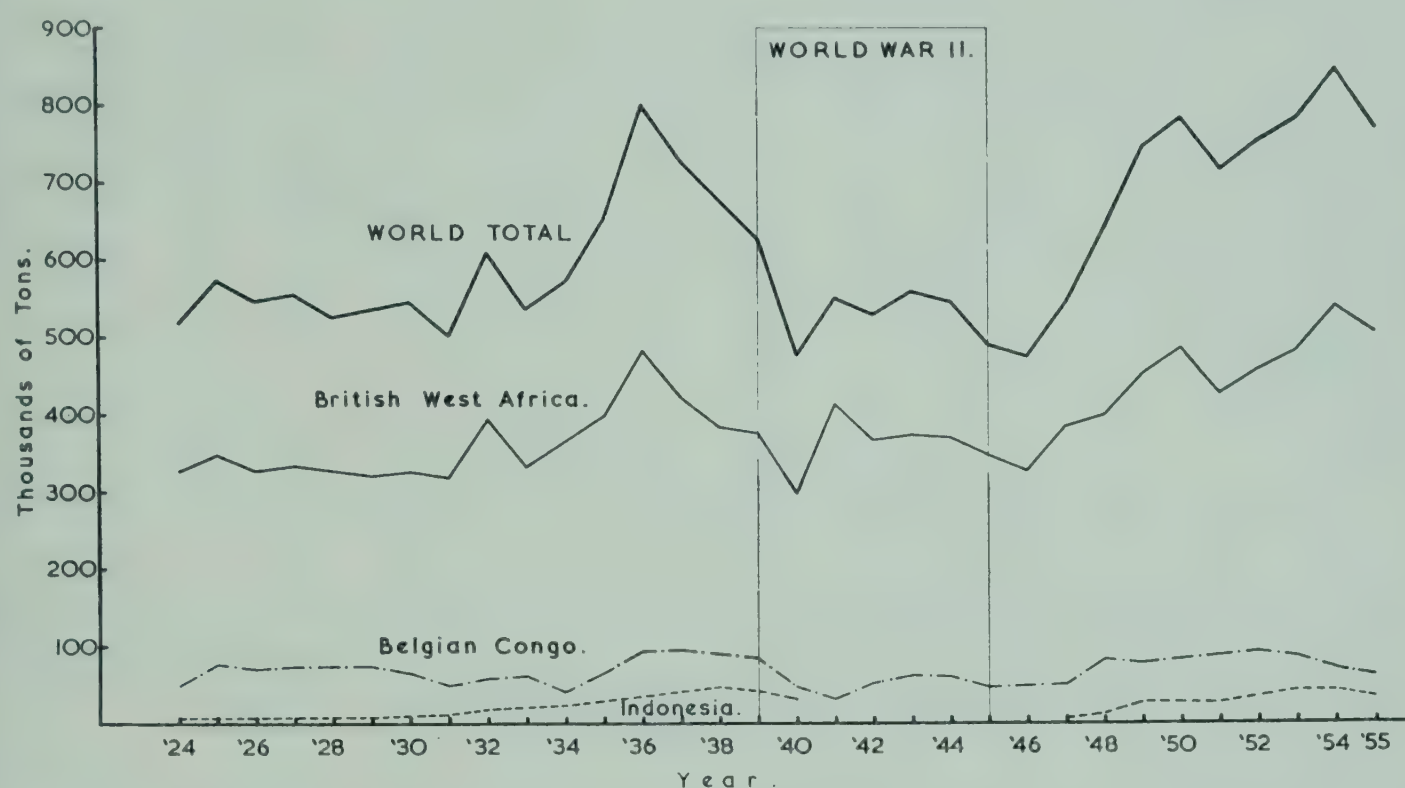


FIG. 2. Annual exports of palm kernels, 1924–55. (Reproduced by permission of the Director of the West African Institute for Oil Palm Research.)

Table I gives details of the exporting areas of palm kernels from 1909 to 1954. Table II gives details of the countries importing palm kernels from 1854 to 1954.

a. Palm Oil

Reference to Fig. 1 shows how remarkably production in the Far East increased between the years 1924 and 1939 as a result of the application of scientific methods in the plantations. Conversely, Nigeria, which produces almost all the output of British West Africa, was precluded from taking any greater share of the expanding world market by the continuation of native methods. Despite war losses, the Far East is regaining its place, and recent plantings may result in a further increase in exports in the next five years.

Exports of palm oil from Sierra Leone and the Gambia are usually non-existent, and there is only a very small amount exported from the Gold Coast.

TABLE II
WORLD IMPORTS OF PALM KERNELS^a

Country	1854	1896	1902	1938	1943	1948	1950	1952	1953	1954	1955
Thousands of long tons											
United Kingdom	—	—	32	133	499	379	455	448	444	303	336
South Africa	—	—	—	5	13	—	—	—	—	—	—
Germany	—	102	160	276	—	4	104	114	107	160	116 ^b
France	0.36	20	8	84	—	75	75	82	130	147	135
United States	—	—	—	11	—	—	—	—	—	—	—
Denmark	—	—	—	24	—	20	19	12	18	27	10
Netherlands	—	—	—	48	—	8	37	38	49	100	106
Belgium	—	—	—	29	—	68	17	1	11	26	25 ^b
Portugal	—	—	—	17	20	11	16	23	14	22	21 ^b
Czechoslovakia	—	—	—	14	—	14	—	—	—	—	°
French Morocco	—	—	—	—	°	2	1	—	2	°	°
Others	—	—	—	25	°	7	5	5	5	°	°
	0.36	122	200	666	532	588	729	723	780	785	749

^a Taken from the following: International Institute of Agriculture; Food and Agriculture Organization of the United Nations, *Monthly Bull. Agr. Econ. and Stat.*; Commonwealth Economic Committee, "Vegetable Oils and Oilseeds," London.

^b Estimated.

° Not available.

The output of plantations in the Belgian Congo continues to increase, and, here again, recent plantings will bear fruit within the next few years.

b. Palm Kernels

The picture given in Fig. 2 shows a very different state of affairs, partly because the botanical form of the fruit in the various areas is different and partly because of the differing economic relationship of palm oil and kernels.

British West Africa remains the supreme exporter of kernels, with Nigeria in the first place, a smaller contribution coming from Sierra Leone, Gambia, and the Gold Coast.

The plantation areas of West Africa, Indonesia, and Malaya make a much smaller impact on the trade, as not only is the proportion of kernels in the fruit being reduced to increase the production of pulp oil, but there is also a local oil milling industry in Malaya using about a third of what kernels are produced. Conversely, in West Africa much

of the palm oil is consumed locally and virtually all the separated kernels find their way into the export market. They require too much capital investment in plant for crushing to make them of value to any but a well-industrialized community.

4. Relative Values of Products

Relative values of products of the oil palm are given in Table III. It is clear that, although palm oil is always of greater economic im-

TABLE III
RELATIVE VALUES OF PRODUCTS OF THE OIL PALM

Type of palm	Portion of weight of fruit bunch		Yield per acre ^a		Value of products per acre ^b			Relative value of products	
	Extract-able palm oil (%)	Palm kernels (%)	Palm oil (lb.)	Palm kernels (lb.)	Palm oil (\$)	Palm kernels (\$)	Total (\$)	Palm oil/kernels	Palm kernel oil ^c /palm kernel meal
High-yielding									
Dura	16	5-6	1400	500	140	30	170	4.7	3.0
Tenera	22	3.5	1700	270	170	16	186	11.3	
Pisifera ^d	30-45	1	Not known		Not known			50-75	

^a On a basis of 55 trees per acre, per annum yield. These values are for Nigeria plantations. Yields in Sumatra may be double this figure.

^b Calculated on basis of palm oil at 10 cents per pound and palm kernels at 6 cents per pound.

^c Calculated on basis of palm kernel oil at 11 cents per pound and palm kernel meal (6% oil) at 3.5 cents per pound.

^d Fruiting Pisiferas are very rare in nature and cannot yet be propagated successfully.

portance, the actual relationship between it and the kernels is widely variable. In a fluctuating market, no precise figures can be given, but experience over the past few years indicates a ratio of value of palm oil to palm kernels of about 10 to 6.

III. BOTANICAL INFORMATION

1. The Tree

The commercial oil palm (see Fig. 3) is the *Elaeis guineensis* (Jacq), a typical member of the *Palmae* most closely related to the American oil palm, *Corozo oleifera*. The tree in its natural state grows

to a height of some 60 feet to get to the light and is characterized by its vertical trunk and the feathery nature of its leaves. It starts bearing fruit some 15 to 20 years after germinating and may have an average life of 50 to 60 years.

In plantations, the trees are grown properly spaced to encourage slow growth which permits easy harvesting, and they come into bearing about 4 years after planting. Normally, they are replaced at the



FIG. 3. Oil palm tree, 7 years old, showing how the fruit bunches form between the trunk and base of the leaf stems; these leaves are known as fronds.

age of about 25 to 30 years when they reach a height at which harvesting becomes too expensive.

The leaves grow in a continuous whorl at the top of the tree, and the fruit grows in bunches at the junction of the leaf and the stem of the tree. Each bunch weighs some 40 lb. and will contain some 1200 to 1500 individual palm fruits which are typical drupes. The plant is monoecious, having both male and female inflorescences growing on the same tree. Male and female flowers do not occur at the same time or according to any strict time schedule so that cross-pollination from one tree to another is necessary to produce fertile seed.

2. The Fruit

The fruit, shown diagrammatically in Fig. 4, consists essentially of (1) a soft, thin outer skin, or pericarp, of a reddish orange color when ripe; (2) a pulpy, fibrous layer, or mesocarp, which contains the palm

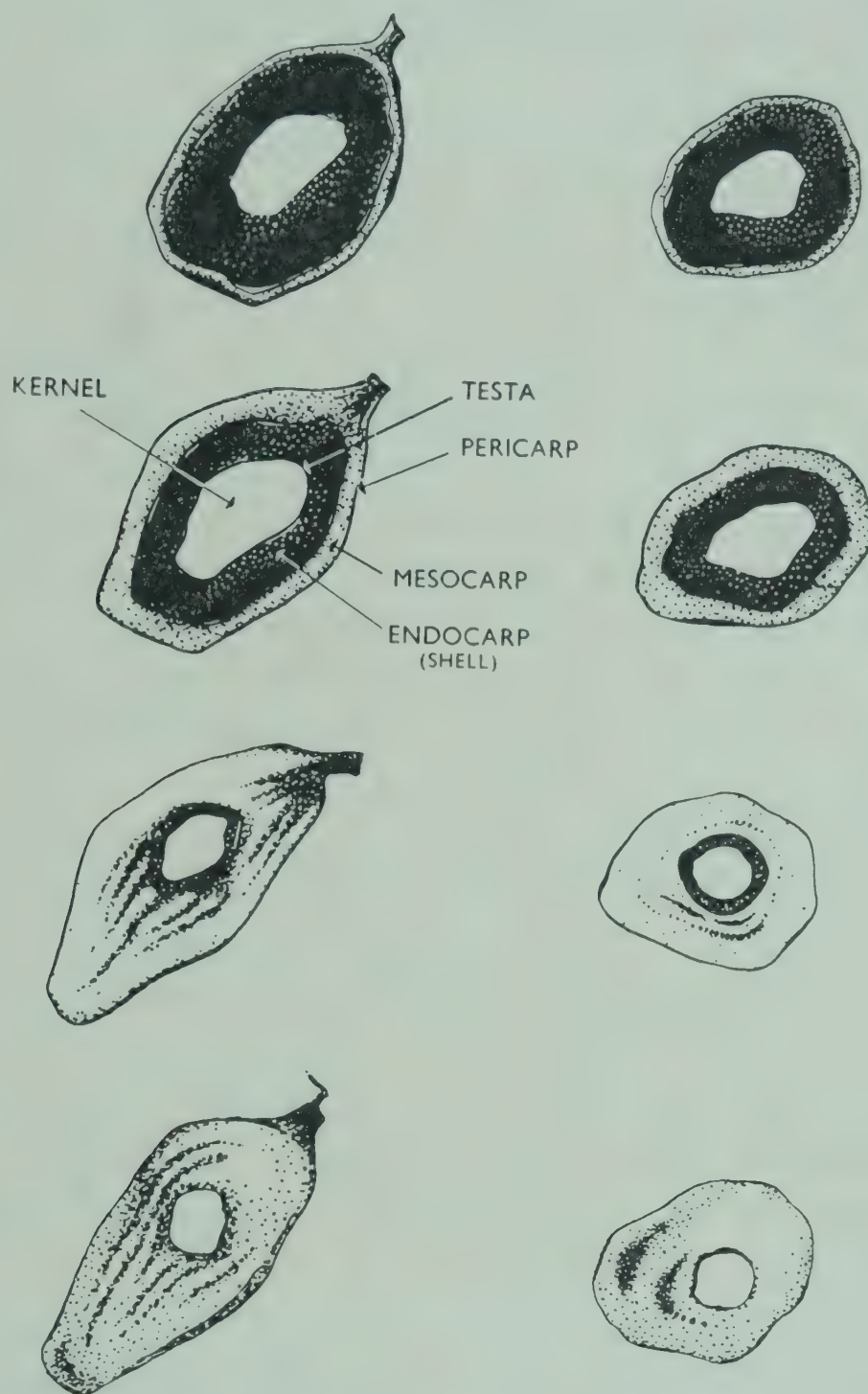


FIG. 4. Diagram of the four major forms of palm fruit, showing, from top to bottom, *Macrocarya*, *Dura*, *Tenera* (the *Dura-Pisifera* hybrid), and *Pisifera*. (Reproduced by permission of the Director of the West African Institute for Oil Palm Research.)

oil; (3) an inner shell, the endocarp; and (4) a kernel which has a firmly attached black testa and contains approximately 49% of an oil, similar to coconut oil, and 8½% of protein.

The fruit can be divided into various types distinguished by the color of the skin. By far the most important in commerce is *Nigrescens*, or Ordinary, which is black until the time of ripening when it becomes orange. There are, however, a number of other types: *Virescens*, or

Green, which as the name implies has fruit which is green until the time of ripening when it becomes orange; Mantled; and White, which is very rare in nature. More important than the types of fruit are their forms which are distinguished by relative amounts of mesocarp, shell, and kernel. The variety in which these forms appear is very great indeed, but they can broadly be divided into four groups, which are shown in Fig. 4. These are:

a. Macrocarya, consisting of a very thick shell and thin mesocarp. These are all self-sown and have no place in scientific plant breeding. Widely scattered in West Africa, they are principally found in Sierra Leone and areas of Nigeria.

b. Dura, which is the most common in the natural palm groves. This has a thick shell but a thicker mesocarp than *Macrocarya* and a smaller proportion of kernel. A particular type of *Dura*, known as Deli, has been almost exclusively the source of all palm products from the Far East. It has an important place in all breeding programs (see later) and lends itself to selection for high yields.

c. Tenera, which has a much thinner shell and a thicker mesocarp; consequently, its oil yield is higher than either of the two previously mentioned. This is the *Dura-Pisifera* hybrid, toward the production of which plant breeding programs are directed.

d. Pisifera, with no shell at all and often no kernel, but a high oil-yielding mesocarp. It is, however, very rare to find a *Pisifera* tree which is capable of producing fruit. The female flowers are usually sterile, and if the trees produce fruit bunches at all they very often start to go rotten before ripening. A few fruiting *Pisifera* trees do exist which are capable of giving high oil yields. The primary commercial interest in this type lies in its ability to produce *Tenera* seed when cross-pollinated with *Dura* trees.

Plant breeding trials (3*a*) have given the following approximate rules for cross-pollinating the trees, the female plant being mentioned first: *Dura* by *Dura* produces 100% *Dura*; *Dura* by *Tenera* produces 50% *Dura* and 50% *Tenera*; *Dura* by *Pisifera* produces 100% *Tenera*; *Tenera* by *Tenera* produces *Dura*, *Tenera*, and *Pisifera* in the ratio of 1 to 2 to 1.

The importance of the *Pisifera* can be clearly seen, as essential to the production of seed of the desirable *Tenera* type.

Trees for breeding are chosen for their high yielding qualities and then selectively cross-pollinated by well-established methods (5). The main difficulty lies in the selection of *Pisifera* trees with high yielding characteristics. A few fruiting *Pisifera* do occur, and attempts are still being made to produce viable *Pisifera* seed from them. If this were possible, the increase in yields per acre would be enormous.

IV. COMPOSITION OF KERNELS AND MEAL

Palm kernels appear to vary little in composition according to type and form; typical analysis of the composition of dry seed is given in Table IV. After the removal of oil, the resultant meal will vary in

TABLE IV
TYPICAL COMPOSITION OF DRY PALM KERNELS

Component	Content
	<hr/> %
Oil	52
Protein ($N \times 6.25$) ^a	8.8
Extractable non-nitrogen	23.6
Cellulose	5.2
Ash	2.0
	<hr/> I.U./g.
Vitamin B ₁ ^b	1.0
Vitamin B ₂ ^b	0.5
Nicotinamide ^b	2.8
Pantothenic acid ^b	0.7

^a This is the conventional conversion factor. A more accurate factor would be 5.8.

^b According to M. C. Malakar and P. Rombauts, *Oléagineux* **5**, Suppl. 1, 12 pp. (1950).

TABLE V
TYPICAL COMPOSITION OF SCREW-PRESSED MEAL

Component	Content
	<hr/> %
Oil	6.5
Water	11.5
Protein ($N \times 6.25$)	18.7
Extractable non-nitrogen	50.0
Cellulose	11.0
Ash	4.0 (Ca, 0.36%; P, 0.60%; Ca/P, 0.60)
	<hr/> I.U./g.
Vitamin B ₁ ^a	1.3
Vitamin B ₂ ^a	0.56
Nicotinamide ^a	5.1
Pantothenic acid ^a	1.4

^a According to M. C. Malakar and P. Rombauts, *Oléagineux* **5**, Suppl. 1, 12 pp. (1950).

composition to some extent with the processing method employed. A typical composition of a screw-pressed meal is given in Table V. According to Malakar and Rombauts (6), a preferable method of expressing the content of non-oil and non-protein materials is in terms of

assimilable and indigestible glucosides rather than as extractable non-nitrogen and cellulose. Alternative expressions for composition of a solvent-extracted meal would be:

Component	Content %
Water	12.2
Protein ($N \times 6.25$)	15.9
{ Extractable non-nitrogen	55.3 }
{ Cellulose	13.3 }
or	
{ Assimilable glucosides	35.5 }
{ Indigestible glucosides	32.5 }
Ash	3.4

There are few references in the literature to the amino acid composition of the protein in palm kernel meals, the analyses available being given in Table VI (6-9). The method of Mitchell and Block (10) of comparing the amino composition with that of the protein of whole egg has been applied to an arithmetical mean of the available analyses. This indicates that methionine is the limiting essential amino acid in palm kernel meal, when considered as a source of protein for non-ruminants. [See also Malakar and Rombauts (6).] Of course an "arithmetical mean" has limited meaning when a variety of analyses on different meals, are being compared.

There are wide variations in the total crude protein content of the samples which are unexplained; analyses of a large number of samples of commercial solvent-extracted meal show an average content of 19.6% crude protein with a standard deviation of 0.6%.

Palm kernel meal must be regarded as a low-protein meal, but its content of essential amino acids together with a favorable calcium to phosphorous ratio enables it to make a valuable contribution to the protein build-up of a compound animal food.

V. METHODS OF PROCESSING

1. Processing Palm Fruit to Obtain Palm Oil and Kernels

As in cultivation, so in processing there are two distinct industries, native and plantation, which provide palm oil and palm kernels.

The native method, still the source of the major part of the world's supplies, is at present undergoing some transformation. There are the age-old methods in which the fruit is stripped from the bunches and

TABLE VI
AMINO ACID COMPOSITION OF PROTEIN IN PALM KERNEL MEAL

Amino acid	Source of sample				
	Hot extracted ^a	Various ^b	Kibbled press cake ^c	Hot extracted ^c	Screw- pressed cake ^d
Calculated as g./16 g. N					
Alanine	—	4.3	—	—	—
Arginine	7.1	11.0	16.2	17.2	13.3
Cystine	1.7	1.7	—	—	—
Glycine	—	4.1	—	—	—
Histidine	—	1.8	1.8	1.8	1.6
Isoleucine	—	—	4.6	5.0	4.0
Leucine	5.5	—	7.6	8.3	6.4
Lysine	3.8	6.7	3.6	4.7	3.4
Methionine	1.5	1.2	2.7	2.3	2.1
Phenylalanine	—	—	4.4	4.8	4.3
Threonine	—	—	3.7	4.0	3.1
Tryptophan	1	1.7	0.8	0.8	1.0
Valine	4.4	—	6.2	6.4	5.4
Crude protein (N × 6.25), %	17	—	15.6	13.3	19.2

^a M. C. Malaker and P. Rombauts, *Oléagineux* **5**, Suppl. 1, 12 pp. (1950).
^b N. Baudoin, *Oléagineux* **5**, 241 (1950).
^c R. W. Carroll, The Quaker Oats Co., private communication; by method of L. M. Henderson and E. E. Snell, *J. Biol. Chem.* **172**, 15 (1948).
^d C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).

allowed to soften by fermentation with a resultant rise in free fatty acid content of the oil. It is then cooked in water until the fibrous outer layer becomes a soft pulp and oil can be skimmed from the surface of the water. After this, the pulp may be squeezed to obtain more oil and the nuts can be separated by hand. The nuts are finally cracked by the women and children, using stone hammers, and collected for shipment. The revenue from the sale of the kernels is by strong tradition the property of the women, and any attempt to change the old order has met with surprisingly active and sometimes violent resistance. This process leaves the kernels entirely unaffected but produces an inferior quality of palm oil containing a high percentage of free fatty acids.

A recent development has been the widespread distribution of hand presses to native oil producers which make possible the production of

palm oil without any need to resort to the softening by fermentation process. This has resulted in a marked improvement in the average quality of native-produced oil.

In the plantations, where quality of oil is held paramount, the first concern is to transport the fruit bunches as rapidly as possible to the oil mills where they are immediately steam-sterilized to minimize fermentation. After sterilization under pressure at 120° , the fruit bunches are fed to a thresher which consists of a horizontally revolving cylindrical cage, or a beater, which removes the fruit and separates it from the stalks. In smaller mills the stripping may be done by hand, but the inevitable delay in processing bruised fruit results in production of an oil of higher free fatty acid content. The fruit is then conveyed to a digester kettle where it is heated to a maximum of 95° ; there the fruit is reduced to a pulpy mass of oil, fiber, nuts, and water.

The palm oil may be extracted from the mass in one of two machines.

a. Hydraulic Press

This is a form of cage press with a ram operating in a perforated cylinder. Pressures of 5000 p.s.i. are used, and about 90% of the total oil is extracted.

b. Centrifuge

A basket centrifugal, operating at about 1200 r.p.m., is used. This piece of apparatus, being simpler to operate and install, is used in a number of small "Pioneer" mills associated with the wild palmeries and is able to extract about 85% of the oil. The oil is settled and centrifuged to remove water and dirt and then passes to bulk storage tanks.

The residue from both press and centrifuge is treated in a nut and fiber separator; the fiber is used as boiler fuel, and the nuts are stored before dehulling. The nuts are first graded into three sizes and then cracked by spinning them against a metal ring; grading is necessary to ensure complete cracking without shattering the kernels. The broken shells and kernels are screened to remove dust and dirt, and the nuts are then separated by floating them in a bath of clay slurry or brine. Shells are burnt as fuel, and the kernels are washed and dried by steam-heated hot air. There is a resultant yellow discoloration of some of the kernels which can slightly darken the expressed oil. Finally, the kernels are screened and bagged for export.

A schematic diagram of the sequence of these operations is shown in Fig. 5.

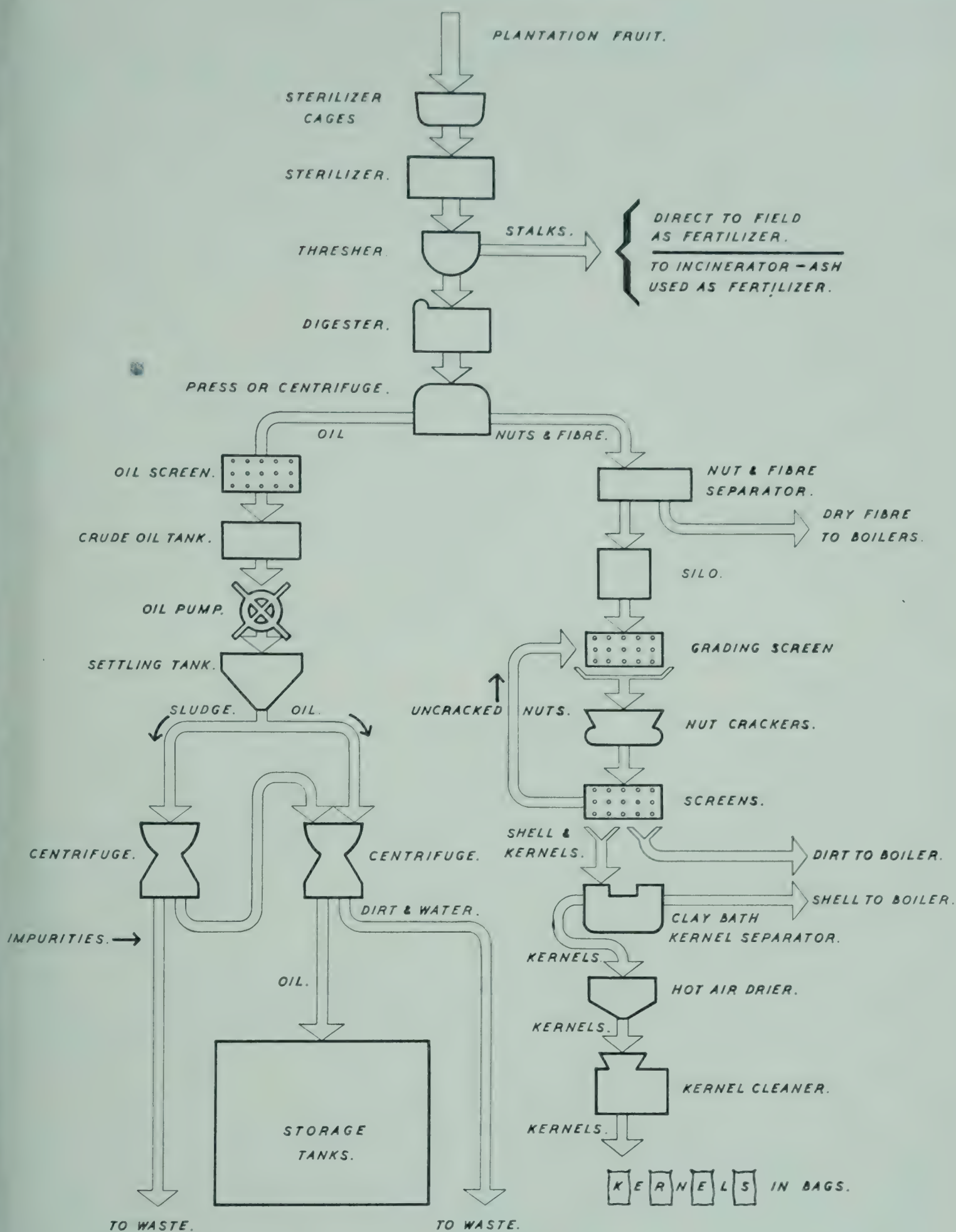


FIG. 5. Palm oil mill, sequence of operations. (Reproduced by permission of The United Africa Company Ltd.)

2. Processing Palm Kernels

There are three commonly used methods of processing palm kernels to produce oil and residual cake or meal in the countries to which they are exported: hydraulic pressing, screw-pressing, and solvent extraction. Hydraulic pressing is becoming obsolescent on account of its high

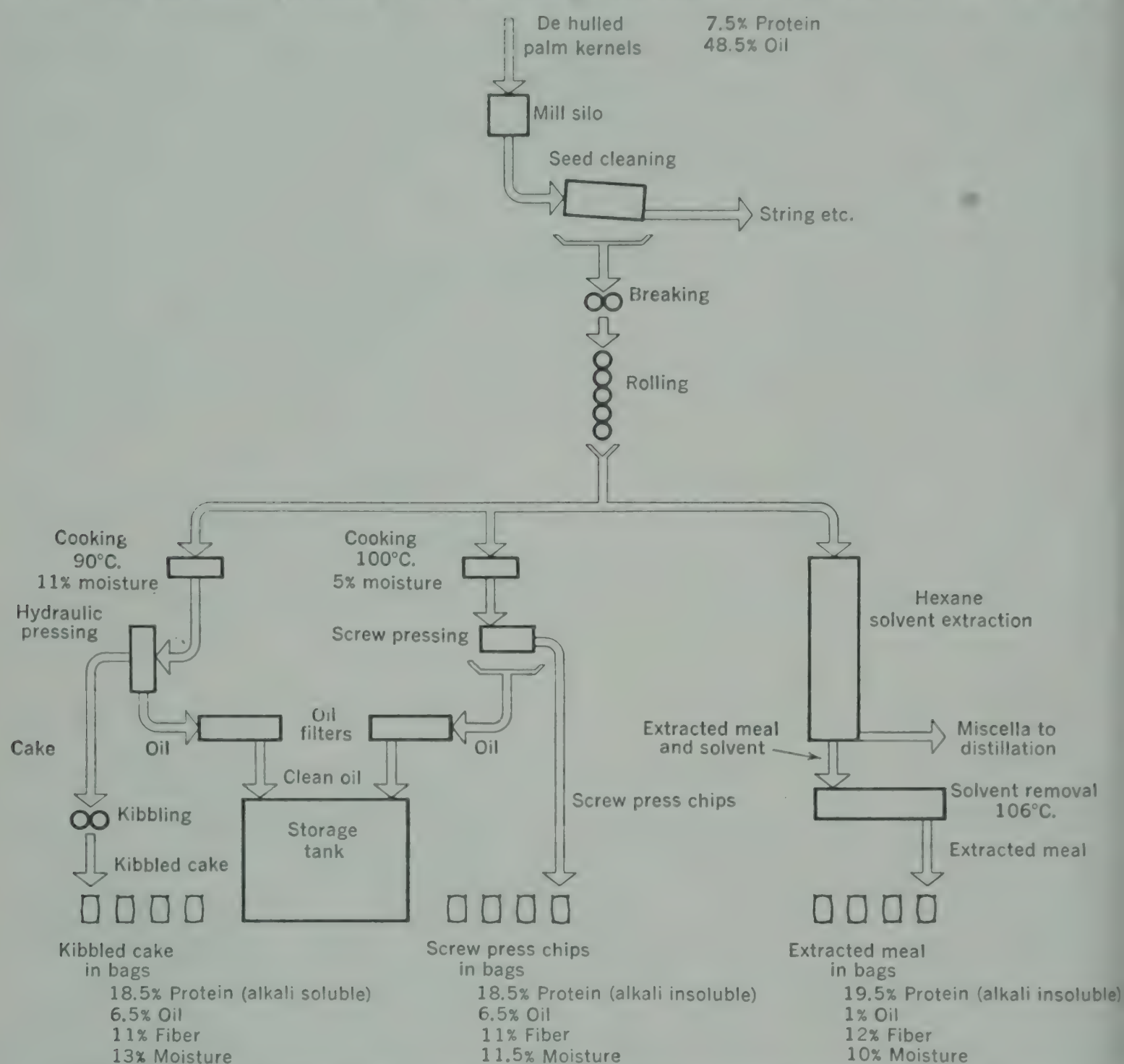


FIG. 6. Palm kernel oil mill, sequence of operations. (Reproduced by permission of The United Africa Company Ltd.)

labor requirements, and solvent extraction has to a considerable degree fallen into disfavor, as solvent-extracted meal finds a poor market as animal feedstuffs. Screw-pressing is, therefore, the preferred process for palm kernels, and its operation can be varied quite widely to produce cakes of either high ($6\frac{1}{2}$ to 7%) or low ($3\frac{1}{2}$ to $4\frac{1}{2}$ %) oil content.

For all three processes the preliminary treatment is approximately the same (Fig. 6). The kernels are unloaded from the ship either in

bag or bulk and stored in warehouses or silos. Thence they are conveyed over magnets to remove tramp iron and through screens to remove large extraneous matter.

a. Rolling

As the kernels are very hard, they are broken in $\frac{3}{8}$ -inch and $\frac{3}{16}$ -inch fluted breaking rolls running at differential speeds, before fine-rolling in five-high Anglo-American rolls.

b. Cooking

When it is to be solvent-extracted, the rolled seed is conveyed to the extractor without any further treatment, but it must be cooked before hydraulic- or screw-pressing. For hydraulic pressing the rolled seed is cooked at a fairly high (11%) moisture content to assist oil release, but for efficient screw-pressing the seed must be dried as much as possible during cooking, without burning (e.g., 3%). There appears to be no advantage to be gained by cooking at high moisture content followed by drying before screw-pressing.

c. Pressing

The oil is not easily removed from palm kernels; hence the maximum power available in a screw press is used with resultant heavy wear on the worm assembly and cage. There is no apparent advantage to be gained in two-stage screw-pressing or prepressing before solvent extraction, the net result of either of these processes being an increase in power and maintenance costs with no better efficiency of oil extraction.

Typical processing details are given in the next section of this chapter dealing with the effect of processing. An outline of the sequence of operations is given in Fig. 6.

VI. EFFECT OF PROCESSING

A brief investigation into the effect of processing on the alkali solubility of the protein has indicated that, as a result of any of the modern commercial processing methods, the protein is rendered almost insoluble in alkali, but the feeding value is not noticeably impaired. More work has to be done, however, to establish definite conclusions.

1. Feeding Value

Little is known of the effects of processing and storage on palm kernels protein, but what information is available suggests that it is comparatively unaffected by any of the usual treatments. The only

investigation appearing in the literature was by Malakar and Rombauts (6), who found no significant difference in amino acid content, B vitamins, or protein between raw kernels, cold solvent-extracted meal, hot solvent-extracted meal, and screw-pressed cake.

Growth rate of rats was unchanged when either of two rations was given, one containing cold-extracted palm kernel meal and the other containing palm kernel meal autoclaved for 30 minutes at 120°, when both rations contained 12% total protein.

2. Alkali Solubility of Palm Kernel Protein

A determination of protein solubility as affected by processing and storage conditions has been made by digesting the meal in caustic soda at pH 11.5 and reprecipitating the protein from the solution with trichloroacetic acid. Although protein solubility has no noticeable relationship to feeding value, it is of value in predicting the physical properties of the meal as they affect ease of pelleting. When applied to the meal produced by any one process, the protein solubility is also a rough indication of the effect heat has had on it. An increase in the non-reprecipitable nitrogen indicates gross heat damage.

a. Determination of Protein Solubility

(1) *Preparation of meal.* Two hundred grams of palm kernels was broken in a kibbler, ground through a laboratory mill, and extracted at room temperature with petroleum ether (boiling point 40° to 60°). The resulting meal was allowed to dry in the air, ground in a pestle and mortar, and re-extracted. The last traces of oil were removed by passing the dry meal from the second extraction through a pair of flaking rolls, and re-extracting. The dry, oil-free meal was finely ground in a laboratory mill on to a 60-mesh screen until it all passed through.

(2) *Method of test.* Ten grams of meal was weighed into a 250-ml. beaker, and 100 ml. of distilled water added. The resulting slurry was stirred continuously, and a 3.5 *N* caustic soda solution added dropwise until a pH of 11.5 which remained constant for 5 minutes was attained. Stirring was continued for a further 30 minutes, the stirrer and electrodes were removed and washed down with the smallest possible amount of distilled water into the beaker, and the whole re-weighed.

The slurry was centrifuged at about 1500 r.p.m. for 5 minutes, filtered through a cotton wool pad, and allowed to stand overnight. Ten milliliters was used to determine nitrogen content by the usual Kjeldahl method, and a further 50 ml. transferred to a beaker and brought to pH 5.0 (± 0.1) by the addition of approximately *N* trichloroacetic acid. The precipitate was removed by centrifuging, the extract clarified by passing through a 54 paper, and the nitrogen content determined.

b. Samples

The samples which have been tested and their properties are listed in Table VII. They are described as follows:

TABLE VII
SOLUBILITY OF PROTEIN IN ALKALI AS AFFECTED BY PROCESSING TREATMENTS

Treatment	Total crude protein (N \times 6.25) (%)	Nitrogen soluble and reprecipitated ^a (% total N)	Nitrogen not reprecipitable ^b (% total N)
Fresh kernels			
Macrocaraya	15.0	73	4.7
Dura	15.0	62	3.6
Tenera	19.4	72	4.0
Pisifera	15.0	59	5.5
Mixed kernels			
Received in United Kingdom	18.2	40	4.4
Stored 2 years	18.6	47	12.1
Simple heat treatment			
Rolled	15.0	42	8.4
Cooked	16.2	36	5.5
Plantation sterilized	17.0	6	5.5
Screw-pressed	20.5	2	5.0
Hydraulic-pressed	18.8	37	5.7
Solvent-extracted (batch process)	20.5	1	5.0

^a A measure of protein—extractable by alkali and precipitated by acid.

^b A measure of non-protein nitrogen.

(1) *Fresh kernels.* Fresh fruit bunches of four different types—Macrocaraya, Dura, Tenera, and Pisifera—were obtained. The kernels were carefully removed by hand and extracted with cold petroleum ether (boiling point 40° to 60°), and the meal was reduced to 60 mesh before testing.

Although no conclusions can be drawn from so small a sample, the Tenera had a significantly higher content of total crude protein of 19.4% compared with an unusually low figure for the others of 15%. The maximum amount of protein soluble in alkali does not exceed 75%.

(2) *Native-produced kernels, fresh and stored.* Kernels as received in the United Kingdom and others after two years' storage were cold-extracted and analyzed. There was no indication of any reduction in solubility, although the non-precipitable nitrogen was unusually high in extracts from stored kernels. This might indicate some breakdown of protein during storage.

(3) *Effect of simple heat treatment.* Kernels as received in the United Kingdom of 6% moisture content were cracked and rolled in five-high Anglo-American rolls at a maximum temperature of 55° and cooked in a screw-press kettle to 92°, with only slight loss in moisture. These were compared with "plantation" kernels which were sterilized according to commercial practice at 120° before digesting, separating, and drying by hot air at 138°.

The solubility of the normal run of commercial kernels, of which the majority are native-produced, was little affected by the rolling and cooking process, but the sterilizing of the "plantation" kernels reduced their protein solubility to about 6%.

(4) *Effect of screw-pressing.* Kernels as received in the United Kingdom were cracked and rolled at 7% moisture content at a temperature of 35°. They

were then partially dried in an open, steam heated, trough dryer to 5 to 6% moisture content at 50° in 6 minutes. Cooking and drying to a temperature of 100° and 4.0% moisture were completed in a screw-press kettle in 15 minutes.

(5) *Effect of hydraulic pressing.* Kernels as received in the United Kingdom were rolled and cooked in a manner similar to that previously described, to a maximum temperature of 92°. They were then pressed in Albion hydraulic presses, and the cakes were cold-extracted and analyzed. The solubility was found to be little lower than that of the untreated kernels.

(6) *Solvent extraction.* Kernels as received in the United Kingdom were solvent-extracted in a batch-type plant with hexane. The kernels were cracked and rolled as was done in the hydraulic-pressing procedure and were then solvent-washed to an oil content of 1.4%. Desolventizing was carried out with live steam at 30 p.s.i. for 30 minutes, the moisture content of the meal increasing to about 15%. The protein was rendered insoluble, but the non-precipitable nitrogen was not increased.

VII. USES

Palm kernel meal is used virtually exclusively as animal feed, for which it has probably been given since the beginning of the century.

Most of the available information was published between about 1909 and 1945, and the majority is of German origin. During the two World Wars the meal became an important feedstuff because of its availability, relative cheapness, and high nutritive value. Some pig farmers were dissatisfied, however, when they were forced to feed it in place of the usual cereal ration (11). After World War II, commercial interest in its use as a high percentage of the total ration declined, and since 1945 very little work on this meal has been published.

Palm kernel meal has the disadvantage of being gritty and dry in texture, and it is not readily accepted by all types of stock (12). Opinions differ as to its palatability, but in Europe solvent-extracted meal is considered to be particularly unpalatable. It has therefore been blended with well-liked foods, such as molasses, and fed in gradually increasing proportions (13). In Europe it is used largely for feeding cattle, although it can be fed to pigs, horses, and poultry. It has been claimed to be among the most digestible feedstuffs for sheep (14), but against this some authorities state that the digestibility of the crude fiber is only 44.8% (15).

It is a general opinion that palm kernel meal is superior in food value to undecorticated cottonseed meal (14-17), and one report states that it is about equal to wheat bran for dairy cows (18).

1. Dairy Cattle

It has been suggested that the specific effect of palm kernel meal in the dairy ration is to increase butterfat production and percentage.

This has been shown in many experiments, but there is also evidence that the milk yield is adversely affected. Such beneficial action of the meal depends on its oil content, but the individual response of the cow is very important and can override the effects of fat level in the feed. The oil content normally comes between 0.5% in an extracted meal and 7.0% in a pressed cake: Keseling (19) suggests that in order to achieve any beneficial effect meal or cake containing at least 6.0% oil should be fed.

Meals of different oil contents were investigated by Bühner and Fissmer (20). Groups of cows were fed either 2.5 kg. per head per day of palm kernel meal (0.15% oil), or the same quantity of cake (5.54% oil), in comparison with a control ration containing an equivalent amount of groundnut and soybean meal. The low fat diet had no effect on the composition of the milk, but the high fat diet increased the butterfat by 0.3%. Cows on the latter diet received 138 g. more fat than the controls and produced 38.9 g. more fat in the milk, that is, 28.2% of the former was converted into butterfat. This work was reported during World War II, and, in view of a scarcity of fat, it was decided that the conversion factor was unsatisfactory and complete extraction of the oil during processing was recommended.

Brouwer (21) fed palm kernel meal containing 6.7 to 7.2% oil at a rate of 2.4 kg. per head per day. Butterfat production was increased by more than 10%, and butterfat by about 0.4%. The effect lasted for the whole experimental period of 8 weeks. In another trial (22) it was found that the changes in yield and composition of the milk were not great, but that the consistency of the butter was improved and the iodine number of the butterfat was reduced by 4.5 units.

Small but significant increases were observed when a change was made from cake containing 1% oil to that containing 5% oil. The cake formed 60% of the production ration, and the higher fat level resulted in an increase of 0.15% in butterfat (23).

Völtz (24) and Vogel (25) also showed that palm kernel cake considerably increased butterfat production and butterfat percentage.

A minimum level of 2 kg. of palm kernel cake per head per day was suggested by Kronacher *et al.* (26). Palm kernel cake (oil content not given) was fed at levels of 2.1 to 3.9 kg., and the average increase in butterfat was 0.4%. The actual increase was determined more by the individual response of the cows than by the level of feeding, provided that a minimum quantity of meal was given. The milk yield was unaffected.

The wide variation of response among individual cows was also shown by Schmidt *et al.* (27, 28), who used mixtures of palm kernel and coconut cake. An increase of 10% of the butterfat content was obtained with levels of 1.5 kg. of mixed cake, without affecting the milk yield; an increase of 16% was obtained at the 3-kg. level, but the milk yield was decreased by 5%. Further increase in the oil cake level, up to 4.5 kg., decreased butterfat production. Results were greatly affected by the individual response of the cows.

Evidence of decreases in milk yield was given by Honcamp *et al.* (29, 30). When rations containing 1.5 to 2 kg. of palm kernel cake (oil content not given) replaced groundnut meal and crushed maize, a definite, though not

substantial, decrease in milk yield was observed. Extracted palm kernel meal, on the other hand, did not affect butterfat production or percentage, whereas palm kernel cake containing 5% or more oil raised the production of butterfat; the higher the oil content, the more pronounced was the effect. Commercial cakes containing 6 to 7% oil, in rations, raised the butterfat by 0.22%. Cakes of high oil content (11.5 to 29.6%) in similar circumstances did not give uniform response but raised the butterfat content by 0.34 to 0.55%. The response of individual cows to these rations was variable, and in extreme cases the products richest in oil had no effect, whereas in other cases the extracted products had a marked effect.

Reports from Cornell University (31, 32) stated that the increases in butterfat produced by substituting 10% palm kernel meal for coconut meal were very slight; the increase over 5 weeks was only 0.08% and too small to be of practical importance. The permanency of the effect was questioned.

Other reports stated that palm kernel meal had no effect on the richness of the milk (33).

2. Beef Cattle

The meal is suitable for fattening cattle at levels up to 4 lb. per head per day (34).

3. Pigs

Palm kernel meal was available to pig farmers during World War II and caused some concern because of its poor palatability and high fiber content. It was contended that the extracted meal was eaten so reluctantly that the rate of live weight increase became incompatible with profitable feeding; the high percentage of crude fiber also depressed digestibility and consequently lowered the nutritive value (11).

In 1916 Crowther (35) experimented with extracted palm kernel meal in rations for pigs between 98 and 133 lb. live weight. The meal was fed initially as about one-fifth of the ration, and gradually increased to about two-sevenths of the ration. Further increases caused scouring, but up to this level it could be used safely. In comparison with the unextracted cake, however, it gave considerably poorer results in terms of live weight gains.

Mackenzie and Fleming (36) reported that pigs could be weaned on rations in which middlings were replaced by palm kernel meal reinforced with one-fortieth of its weight of blood meal and steamed bone flour. The meal could also be used in finishing rations constituting as much as 62% of the total.

These results were confirmed and extended by Woodman and Evans (11). They made farm-scale feeding trials to test the suitability of the extracted meal fed at different levels in place of fine bran. The percentage of meal was increased from 17 to 60 as the live weight increased from 50 lb. to 150 lb. and over. The optimum feeding level lay between 30 and 35% of the total ration. Below this level the unpalatable nature of the feed was masked by the other ingredients and the effect of the meal was at least equal to that of fine bran. Only small quantities could be safely fed to newly

weaned pigs, and at 50 lb. live weight the amount could be increased to 15 to 20% of the total ration. At all levels it was important to accustom the animals to the new feed.

Carstensen (37) found that a mixture of palm kernel meal and soybean meal was as efficient and cheaper than fish meal for fattening pigs from about 80 lb. live weight. Its suitability for younger pigs was not investigated.

The effect of palm kernel meal on the composition of bacon fat was studied by Willcox and Cranfield (38); the only changes were a slight rise in saponification number and also in melting point. This did not follow the increases in palm kernel meal in the rations, and the analytical data did not explain the changes in the quality of the bacon and the consistency of the fat.

4. Sheep

It has been claimed that palm kernel meal is one of the most digestible feedstuffs for sheep (14), but it is also reported that only 44.8% of the crude fiber is digested (15).

5. Fowl

Palm kernel meal is said to be more palatable to fowl than to other types of stock. There is, however, very little information about it. In one case the meal was used during a shortage of maize; it was an excellent feed and capable of replacing wheat middlings (pollards) in rations for growing chickens and laying hens (39). Temperton and Dudley (40) found that satisfactory egg production could be maintained for 9 months when palm kernel meal replaced wheat middlings in rations for battery hens. The meal was as valuable as soybean meal for day-old chicks when fed as 8% of a ration containing 8% of codfish meal (41).

6. Horses

Palm kernel cake can be of value in rations for horses when broken and mixed in well, and fed at levels of 0.5 to 1.0 lb./day, depending on its protein content. It has been used as a partial substitute for oats at levels of 2 lb./day for working horses and is said to be especially good for horses which are out of condition. No difficulty has been experienced in persuading horses to eat the feed, though it invariably causes considerable salivation (34).

VIII. TRENDS

It can only be emphasized here that controlled trends are consequent on the economic incentive to produce the highest yield of palm oil per acre at the expense of palm kernels. There remains in Africa, however, a vast native industry in which palm kernels play an important role. Some disturbances in Nigeria following the attempted

introduction of "Pioneer" mills in native palmeries have indicated that there is little likelihood of any change in the near future of the method of preparing kernels for export which has remained virtually unchanged over the centuries. Indeed, events in the plantation areas may cause palm kernels to assume even greater economic importance in the native palm industry.

Recent plantings in the Belgian Congo primarily of Tenera palms coupled with further extensive planting in the Far East can be expected in five to ten years' time to give rise to keen competition in the palm oil market. Although the plantations will have an advantage in such a market, the introduction of hand presses has permitted the native producers to enter it and at the same time maintain the production of high-quality kernels.

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CHAPTER 25

ALFALFA AND OTHER LEAF MEALS

C. RAY THOMPSON

I. INTRODUCTION

The production and use of alfalfa and other leaf meals is a recent development in the practice of animal feeding. Almost since man first domesticated herbivorous animals, forages have been cut and dried for feeding to livestock when pasture was scarce, but the use of finely chopped or ground forage plants awaited the development of a mixed feed industry. Most of this development has occurred during the past fifty years.

Pioneer nutritionists (1) observed that green forages supplied some nutrients such as provitamin A (carotenoids) abundantly, whereas sun-dried material was relatively poor in these nutrients. Efforts to prevent these losses of labile factors led to the finding that rapid dehydration provided better retention (2). By chopping the forage prior to drying, handling and the actual dehydration process were facilitated. Further grinding of the dried material to a meal resulted in a less bulky product and one which could be mixed readily with other milled feed ingredients.

Feed manufacturers in the early days of this practice utilized a good grade of sun-cured alfalfa as the raw material for production of alfalfa meal, but recognition of the prime importance of vitamin A in the diet of livestock led to the development of a dehydration industry in the early 1930's. Alfalfa was the preferred choice for dehydration because of its high yield per acre, its easy adaptation to many climates and soils, its high content of protein, carotene, and minerals, and especially because it could be cut repeatedly, thus ensuring a steady flow of raw material to dehydration plants over a relatively long period of the year (3).

A number of other species grown in cool, damp climates appropriate to grass production were found to be suitable for dehydration. Timothy, ryegrass, reed canarygrass, orchardgrass, and alta fescue, along with

legumes such as ladino clover and alsike, usually in mixtures, gave high-quality products on dehydration, but yields per acre are usually less than those obtained with alfalfa (4). Many other green materials, such as red clover, white clover, pea vines, and sorghums (5), have been dehydrated successfully; however, because of the inability of most crops to withstand frequent cutting and yet provide a sustained yield throughout the entire growing season, they are not dehydrated commercially. Capital investment in dehydration equipment is too great to allow establishment of a farm operation for one or perhaps two cuttings.

Some areas of the world, such as New Zealand, parts of Australia, France, and the Scandinavian countries, are well suited to production of green forage for dehydration. They also have the necessary mechanization, but the lack of ready fuel supplies has prevented them from establishing a dehydration industry of any size.

II. PRODUCTION AND PROCESSING

Sun-cured alfalfa meal, often a leaf-rich fraction, was the first "leaf meal" produced for mixed feeds in the United States. This was made simply by grinding sun-cured hay coarsely and screening. In such a treatment the leaves were easily shattered and went through the screen while the stems remained above. Production began in the early 1920's and reached 250,000 tons by 1936 (6). It increased to 600,000 tons in 1946 but has declined since, and in 1955 was about 200,000 tons.

Dehydrated alfalfa (artificially dried as compared to sun-cured) was first produced commercially in the United States in 1930 and reached an annual production of about 200,000 tons by 1940. When supplies of vitamin A from fish liver oils were cut off from Japan and Scandinavia during World War II, annual United States production was increased to 245,000 tons in 1943 and 500,000 tons by 1946. Since then production has increased to about one-million tons in 1952 and has fluctuated near this figure ever since (6).

Production of dehydrated grass in Northwest United States and Western Canada is a small but progressive industry. Orchard grass and ladino clover is the predominant mixture grown, but alta fescue, alsike clover, and rye grass are used also. Two, three, and sometimes four species are mixed in a single field. Production figures for the past are unavailable; the entire production of the section was about 25,000 tons in 1955.

A similar industry has grown up in the United Kingdom. Most of the dried green crops are orchard grass (cocksfoot), rye grass, or meadow fescue, with some wild clover or alfalfa. Little pure clover or alfalfa is dehydrated. Production of these crops in 1952 reached about

250,000 tons but has now declined to about 175,000 tons. This country is characterized by having over 1000 small drying plants, each of which produces up to 400 pounds of dry material per hour. This large number is caused by haulage costs which are considered too high if the distance from field to dryer is over one mile (7). Large commercial dryers produce up to one ton of dehydrated product per hour.

Holland and Denmark are reported to produce some dehydrated forage.

III. BOTANICAL DESCRIPTION

The most widely grown alfalfa is the so-called "common" species, *Medicago sativa*, varieties of which have been obtained by repeated planting and natural or planned selection in a given state or area, eventually producing three commercial groups: the individual commons, Turkistan, and non-hardy (8). These are herbaceous, perennial legumes with deep roots, purple flowers borne in loose racemes, and spirally shaped seed pods, each of which contains several kidney-shaped seeds. A distinct taproot is established.

Yellow-flowered alfalfa, *Medicago falcata*, has light- to deep-yellow flowers, many stems, and is quite bushy. It has many fibrous roots. This plant is not of particular economic importance but has been a source of hardiness character for breeding purposes, yielding, when crossed with the commons, the variegated alfalfas, *Medicago media*. Varieties of this species, such as Grimm, Baltic, Ladak, Ranger, Hardigan, and Canadian variegated, are some of the hardiest, highest producers found for northern climates.

Timothy, *Phleum pratense*, because of ease of culture, high nutritive value, hardiness, and palatability, is one of the most important grasses grown for dehydration in temperate climates (9). Reed canary-grass, *Phalaris arundinacea*, is a coarse but vigorous grass well-suited to damp, cool climates which, because of its long growing season, provides good material for processing into leaf meal.

Ryegrasses, *Lolium* sp., yield high-quality forage when cut at an immature stage. Rapid growth during spring and fall aids in providing uniform production when this species is mixed with legumes which produce better growth during summer.

Orchardgrass, *Dactylis glomerata*, or cocksfoot, as it is called in the United Kingdom, is a high-yielding plant well adapted for combination with legumes to give a sustained, high yield. It is less hardy than timothy but thrives on poorer soils.

Alta fescue, *Festuca arundinacea*, is a tall grass (3 to 4 feet), often grown in pure stand which, after establishment, produces heavily

throughout summer months because of a deep root system. Regular harvesting at an immature stage yields material suitable for conversion into a high-quality dehydrated meal.

Ladino clover, *Trifolium repens*, and alsike clover, *Trifolium hybridum*, are usually grown in combination with some of the above-mentioned grasses, the legumes providing a higher protein content. If grown alone, they fail to produce well in fall and spring. Moreover, frequent close mowing may retard regrowth during hot weather.

IV. METHODS OF PROCESSING

Processing of plant materials to produce meals may follow the time-honored practice of mowing, windrowing, and sun-drying. The crop is then picked up by hand and hauled by wagon or truck to a mill where a hammer mill or other grinder is hand-fed to achieve the necessary comminution. The resulting product is handled further in burlap bags. This process is arduous and slow and yields a poor-quality product which is variable in nutrient content and so dusty that workmen object to handling it.

When it was found that rapid drying at relatively high temperatures preserved the much prized carotene (provitamin A) (10), the modified practice became as follows: green material was cut, loaded by hand or hay loader, and hauled to a central point where it was hand-fed into a chopper prior to dehydration. The loading of the long-stemmed plant material into a large mass always resulted in a heavy, soggy, badly matted product which required much hand labor to tear apart before chopping (11). This led to the development of harvesters which now mow the crop, let it fall on a draper, and chop it continuously into lengths of 3 to 5 cm., after which it is blown directly into dump trucks or trailers. This avoids contamination with soil, crop residues, and other foreign material and, most of all, matting in the load. The chopped material is dumped into the hopper of a mechanical feeder which serves to meter the green product into the dryer at a uniform rate—the latter operation being something which was very difficult to accomplish by hand-feeding.

Many types of dehydrators have been designed and employed. Fuel is ordinarily gas or oil because of its convenience, but coal has been used successfully. The temperature to which the material is subjected on entering the dryer ranges from 425° to 1200°C. Even at the highest temperature, green material seems to suffer little charring if it is handled rapidly enough because of the rapid evaporation of moisture. Agitation of the green material is necessary because thick layers of heated

green material quickly "stew," becoming limp clumps, similar to freshly cooked spinach, which are difficult to dry.

Tunnel dryers in which the green material is spread in a porous mat on wirecloth conveyors are used to a limited extent. These employ low temperatures, the hot gases from the furnace having been diluted with cold air prior to blowing through the mat. Dropping from one conveyor to another provides some agitation, but generally this kind of dryer produces a grayer, less-green product than do dryers of other designs because of its inability to prevent "stewing." Drying time is 25 to 45 minutes.

The most widely used dehydrators are rotary, high-temperature drums equipped with internal vanes which continuously lift the green material from the bottom and shower it down through the blast of hot gases from the furnace (Figure 1). The drums are constructed as three concentric shells which provide a "triple pass" of material—the green product enters the center cylinder, travels to the end, returns to near the origin through the intermediate shell, and finally travels to the end through the outermost cylinder. This arrangement conserves heat and allows for a compact unit. The dried product is collected in a cyclone collector while the water vapor is exhausted through a large fan which provides a draft for the burner and serves to convey the chopped product through the system.

The chopped, dry product is dropped from the cyclone collector into a hammer mill which grinds it to a meal, usually about 40 mesh in size. Before or during grinding, 0.5 to 5.0% of vegetable oils or animal fats may be introduced to reduce the dustiness and give a greener color to the finished product. In countries where certain antioxidants are allowed, they can be included with the oil to give a product in which the carotene is more stable during subsequent storage. No difficulties have been encountered with rancidity of fats on leaf meals. Naturally occurring antioxidants probably prevent oxidation of the unstable fats.

The dry meal may be stored and shipped in burlap or paper bags, but because of its dustiness and low density it is often pelleted. This serves to reduce the volume to about 60% of that of the original meal. One alfalfa meal producer in California adds 10 pounds of animal fat containing 0.3 pound of 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (an antioxidant) per ton of meal during hammer-milling to reduce dust and preserve carotene, and then pellets the meal immediately. The stabilized pellets are cooled and stored in one-ton boxes in cooled or refrigerated warehouses until ready for shipment. The pellets are then reground to a coarse meal, 20 pounds of fat per ton is added, and the dark-green, granular, dust-free product is shipped in paper bags.

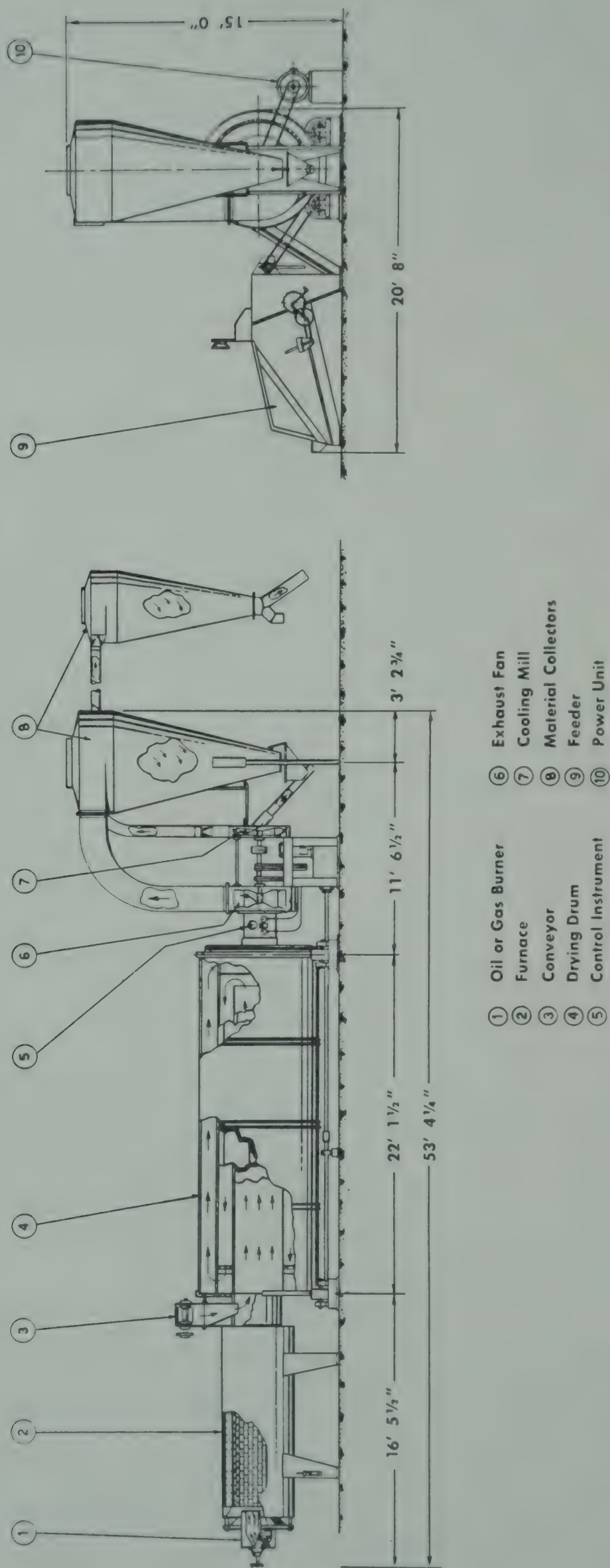


FIG. 1. Cutaway view of high-temperature rotary forage dryer.

Carotene may be preserved by storage under an atmosphere devoid of oxygen (12). This is currently being done with about 200,000 tons of dehydrated alfalfa in the United States. Pelleted material is stored in steel tanks under an oxygen-free atmosphere obtained by use of a special burner. The pellets are usually crumbled or reground prior to shipment.

V. COMPOSITION OF LEAF MEALS

The classical feed-type analyses for protein, fat, fiber, minerals, and nitrogen-free extract are fair indexes of comparative nutritive quality of leaf meals but fail to provide an accurate picture of actual plant

TABLE I
CONSTITUENTS OF ALFALFA

Constituent	Dry weight (%)
Celluloses	30
Protein (nitrogen \times 6.25)	18
Starch, sugar, and pectin	11
Lignin	10
Fats	8
Organic acids	7
Metal ions	6
Tannins	3
Saponins	1.0
Stachydrin	0.25
Betaine	0.45
Chlorophylls	0.4
Xanthophylls	0.04
Tricin (flavone)	0.02

constituents. One has only to consider the free amino acids, alkaloids, chlorophylls, phosphatides, and other nitrogen compounds which contribute to the Kjeldahl nitrogen value to realize that "protein" is an inaccurate designation.

Compilation of isolated data from several sources shows the over-all composition of alfalfa to be somewhat as given in Table I. The content of B vitamins is given in Table II. Alfalfa meal is a rich source of the B vitamins and of as yet unidentified growth factors (13).

The use of ion-exchange resins and paper chromatography has simplified the separation of the organic acids of alfalfa to such an extent that most of them have been determined. E. M. Bickoff and A. L. Livingston of the Western Utilization Research Branch found (unpublished data) that citric, malic, oxalic, and malonic acids comprise 70% of the total, with the remainder consisting of minor amounts of

succinic, fumaric, and several unidentified acidic compounds. The non-amino organic acids make up 7% of the dry weight of alfalfa.

The proteins of alfalfa have been studied to a limited extent. Mitchell *et al.* (14) found that hydrolytic degradation of the proteins

TABLE II
B VITAMINS IN ALFALFA

Vitamin	Amount
	mg./lb.
Choline	400
Inositol	950
Riboflavin	7
Niacin	18
Pantothenic acid	16
Folic acid	4
Thiamine	3
Pyridoxine	6
	γ/lb.
Biotin	150
Thioctic acid	275

TABLE III
NITROGENOUS FRACTIONS OF FRESH, DEHYDRATED, AND SUN-CURED ALFALFA^a

Nitrogenous fraction	Condition of alfalfa		
	Fresh	Dehydrated	Sun-cured
	% of total nitrogen		
Protein	83.4	79.6	72.9
Soluble	16.6	20.4	27.1
Ammonia	0.25	0.26	0.40
Amide	2.7	2.2	2.8
Basic	5.0	8.5	11.7
Amino	9.2	11.6	15.4
Peptide	2.1	2.7	3.1

^a H. L. Mitchell, F. C. Lanning, and R. E. Silker, *Trans. Kansas Acad. Sci.* **53**, 190 (1950).

took place during sun-curing, as is shown by the increases in soluble nitrogen fractions. An example of these data is given in Table III.

Steward *et al.* (15) studied the amino acids of alfalfa. They found aspartic and glutamic acids, arginine, alanine, serine, valine, the leu-

cines, and tyrosine along with the amides glutamine and asparagine, cystine, and small amounts of γ -aminobutyric acid as free amino acids. Combined amino acids were those most commonly found in proteins. Tisdale *et al.* (16) reported that Kingsley (17) found alfalfa to be low in content of sulfur amino acids. Tisdale's studies showed that fertilization of sand cultures of alfalfa with extra sulfate increased the methionine content 50 to 60% over the control in one case and gave 80 to 120% increases in the cystine content.

TABLE IV
ESSENTIAL AMINO ACID COMPOSITION OF ALFALFA LEAF MEAL

Constituent	In sample (%)		In 16 grams of nitrogen (grams)	
Crude protein ^a	19.5 ^b	18.8 ^c	—	—
Arginine	1.0	0.8	4.9 ^b	4.0 ^c
Histidine	0.4	0.3	2.2	1.7
Isoleucine	1.0	0.9	5.2	4.7
Leucine	1.5	1.3	7.9	6.9
Lysine	1.1	1.0	5.6	5.6
Methionine	0.3	0.1	1.5	0.4
Phenylalanine	1.0	0.8	5.1	4.2
Threonine	1.0	0.7	4.6	3.8
Tryptophan	0.4	0.4	2.1	1.5
Valine	1.1	1.5	5.6	4.3

^a Calculated as nitrogen \times 6.25.
^b C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).
^c H. H. Williams, *Cornell Univ. Agr. Expt. Sta. Mem.* **337**, 31 pp. (1955).

Lyman *et al.* (18) and Williams (18a) have analyzed alfalfa leaf meal for composition of essential amino acids, with the results shown in Table IV. Their values for the content of the individual amino acids are in fair agreement with the exception of the ones for methionine. Availability of the amino acids in proteins of dehydrated alfalfa and grass is much lower, however. Ellinger (19) and Carpenter *et al.* (20) have found that the digestibility of leaf proteins for rats and chicks was reduced about one-half after drying. Further information on leaf proteins is given in Chapter 3.

The carotenoids of alfalfa have been studied in some detail (21). Beta-carotene is the only hydrocarbon pigment reported to date to have provitamin A activity. The amounts which occur and the degree of isomerization are shown in Table V. It should be noted that dehydration causes some isomerization of the all-trans to cis isomers, re-

sulting in about 15% less provitamin A potency of the remaining carotene (22, 23). The content of xanthophylls which serve to provide pigments for the shanks, beak, skin, and fat of chickens is high in leaf

TABLE V
 β -CAROTENE STEREOISOMERS OF ALFALFA^a

Condition of alfalfa	Isomers of β -carotene			
	Neo B	All-trans	Neo U	Total
		$\gamma/g.$		
Fresh	10	180	10	200
Dehydrated	64	92	24	180
Sun-cured	6	38	6	50
Relative provitamin A activity of isomers	53	100	38	

^a E. M. Bickoff and C. R. Thompson, *J. Assoc. Offic. Agr. Chemists* **32**, 775 (1949).

TABLE VI
XANTHOPHYLLS OF FRESH ALFALFA^a

Constituent	Amount ^b (%)
Lutein	47
Violaxanthin	32
Neoxanthin	15.5
Zeaxanthin	2
Cryptoxanthin	1
Neolutein	1
Violeoxanthin	0.5
Neovioleoxanthin	0.5
Monohydroxy- α -carotene	0.5

^a E. M. Bickoff, A. L. Livingston, G. F. Bailey, and C. R. Thompson, *J. Agr. Food Chem.* **2**, 563 (1954).

^b The total xanthophylls comprise 0.04% of the dry weight of the material. Figures in this column represent per cent of total.

meals. Table VI gives the analysis of freshly cut alfalfa for xanthophylls (24).

As was shown for carotene, dehydration isomerizes the xanthophylls. Chromatography on magnesia of the xanthophyll fraction from dehydrated material has shown the presence of forty or more isomers arising from the original pigments listed in Table VI.

One group of minor components receiving intensive study is the saponins. Some of these glycosides which have been isolated when fed to chicks cause growth inhibition (25), and when ingested by ruminants may cause bloat (26). Alfalfa may contain as many as ten saponins, several of which are unidentified to date. Which of the saponins it is that causes the unfavorable effects on chicks and ruminants has not been determined. Preliminary studies show, however, that some samples of alfalfa may be tenfold as great in total saponin content as others. This may explain why ruminants pasturing on some fields do not bloat, whereas animals on other fields die from this cause.

No over-all compilation of data on a particular grass is available. Generally, grasses at maturity are about 13% in protein content and contain 200 p.p.m. of carotene (27). Fiber content will vary from 20 to 30%. Analyses indicate, however, that grass cut for dehydration at a much younger stage will contain 15 to 21% protein, 200 to 400 p.p.m. of carotene, 18 to 22% fiber, and about 6 to 8% ash. Grasses generally have about equal carotene content but contain only 60% as much protein as legumes at a comparable stage of development. They are very low or devoid of saponins, which renders them attractive to poultry feeders.

VI. USES OF LEAF MEALS

Leaf meals are used almost entirely as ingredients for mixed feeds. A few attempts have been made to feed them directly, as in the supplementary feeding of sheep and cattle which have been on poor range for an extended period. The material is ordinarily pelleted for this purpose and has given surprisingly good increases in feed utilization with animals feeding on forages which may have been dry for several months. The extra vitamins and minerals improve the adequacy of the diet and also provide a better medium for the ruminal flora, thus further aiding in the digestion of fiber to yield more energy.

About 60% of the total leaf meal production used in mixed feeds goes into poultry feeds, 10% into cattle and dairy feed, 10 to 15% into hog feed, and the remainder into rabbit, dog, and other small-animal feeds. A specially processed alfalfa leaf meal is included at a low level in Pabulum, a widely used infant cereal.

One to five per cent of leaf meal is the level ordinarily fed to young chicks. Levels higher than 5% may cause growth inhibition in some cases, presumably because of the saponin content (28). The high fiber content is also objectionable. Five to fifteen per cent of leaf meal can be used safely with laying hens. Turkeys tolerate even higher levels of leaf meals; levels up to 25% may be given (29). Cattle, dairy, and hog feeds

often contain 10 to 15% leaf meal, and rabbit feed uses 50% or more. Rabbits are reported to prefer sun-cured alfalfa to the dehydrated material.

Uses of grasses and leaf meals for human food are discussed in Chapter 9 and by Pirie (30).

VII. TRENDS

The present trend is toward more processing to produce higher and more uniform quality. Because leaf meal is a minor mixed-feed constituent, the feed manufacturer cannot afford to spend time or effort to improve the product after he receives it. Thus, the primary manufacturer must do all he can to ensure the most uniform product of highest quality if he is to survive. Antioxidants are added to preserve carotene; oils are added to overcome dustiness and improve color; pelleting reduces bulk and dustiness; inert gas storage gives a higher, more uniform carotene content; and extensive blending overcomes variability in quality from lot to lot. Blending has allowed the manufacturer to market a guaranteed product such as one containing 100,000 I.U. of vitamin A per pound, 17% protein, and less than 27% fiber.

The synthesis of vitamin A with the subsequent lowering of its price has had a depressing effect on the price of leaf meals. Research has shown, however, that leaf meal contains many more nutrients such as B-vitamins, minerals, protein, and several unidentified factors so that it is sold, instead, on the basis of its rich spectrum of nutrients. Many feed manufacturers include it as a "protective" feed to ward off unknown or borderline nutritional deficiencies.

The initial announcement of the so-called growth inhibitor in alfalfa reduced the amounts used in poultry feeds, but later studies have shown that 5% or less of most leaf meals will stimulate rather than inhibit the growth of chicks and that 10 to 15% or even 20% of particular lots may not inhibit growth.

From a long-range standpoint, the production of dehydrated leaf meal, or perhaps, more generally, dehydrated forage, will probably continue to grow, especially in times of short supply of animal feeds. Rapid dehydration results in 10% or so more actual pounds of feed produced per unit of land because of respirational losses, leaf shattering, weathering, and bacterial destruction of nutrients which arise from sun-curing. The quality of the dehydrated product is also much higher because of the preservation of labile nutrients. As mechanization of agriculture increases, more and more of the green products may be expected to appear.

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CHAPTER 26

PEAS AND BEANS

I. DESCHAMPS

I. INTRODUCTION

Peas and beans are beyond doubt one of the outstanding sources of vegetable protein available to man. Practically everywhere on earth, and as early in history as may be traced, one will find members of this group of flowering plants rendering a direct service to human beings. They supply a valuable complementary food for the daily needs of man and domestic animals and play a major role in the improvement of soils (1, 2).

These legume seeds* are generally characterized by a relatively high content of protein and an even greater proportion of carbohydrates; their oil content is usually low. Some few members, however, are unusually rich in oil to the point that they may be considered as atypical. This is true for peanuts and soybeans, which depart from the common pattern of composition of most legume seeds. In the present review we have excluded these atypical members; they are discussed elsewhere. (See Chapters 14 and 16.)

Although no better heading than "Peas and Beans" could have been suggested for this chapter, we must admit the possibility of confusion resulting from poor usage of these terms. The words "pea" and "bean" have been used so broadly that they are often even interchanged or applied to seeds of an entirely different botanical origin. Throughout this discussion we shall refer, of course, to legume peas and beans within the accepted botanical classification.

The botanists and horticulturists will readily recognize differences between beans and peas. Beans have a herbaceous stand and bear rather flat seeds; the leaves are large and trifoliate. Their habit of growth varies from distinctly bushy to trailing and twining. The cotyledons of beans develop above ground during germination (epigeal).

* The term *pulse* is often used to designate these seeds.

Peas bear definitely round seeds and exhibit trailing or vining habits of growth. They possess small leaflets (3, 4).

II. BOTANICAL CLASSIFICATION

Among the numerous families of dicotyledonous flowering plants the Leguminosae is in many respects the outstanding tributary; it ranks as the second largest family, with some 12,000 recognized species (5). The great majority of useful seed-yielding legume plants derive from one of the key subfamilies, the Papilionatae. Ten tribes with more than 300 genera are in this subfamily (5), all possessing some common basic characteristics. Four of the botanical tribes which make up the Papilionatae are of special significance, as all common peas and beans belong to several of the genera in these tribes. The Hedysareae, with 43 genera, includes 950 species (5) and contains the well-known peanut (groundnut) plant (*Arachis hypogaea*). The Viciaeae, with 6 genera (5), includes some of the best-known members of economic importance, species found in the genus *Vicia* (120 species), *Cicer* (14 species), *Pisum* (6 species), *Lens* (6 species), and *Lathyrus* (100 species). The Genisteae tribe contains 43 genera which include 950 species (5). A section of this tribe, the Spartiinae, includes in its 9 genera an important group of species known as the *Lupinus*. The Phaseoleae, with 47 genera, includes among them the genus *Phaseolus* as the largest one, with 150 species; other members are in the genera *Dolichos*, *Canavalia*, *Glycine*, and *Stizolobium*. The *Phaseolus* genus will cover the common beans, and the *Glycine* genus such important members as the soybean.

III. PRODUCTION

Of the numerous species of legumes known to the botanist, relatively few are cultivated. Furthermore, we will limit the discussion to the common seed-yielding species; hence all legume members currently grown for green forage and for soiling will be excluded (1, 6).

Five rather homogeneous groups of legume seeds constitute by far the major crops. The chick peas, the peas, the broad beans, and the lentils constitute four major groups, all of which are included within the Viciaeae. The common beans (*Phaseolus*) form another large group of important species and varieties within the Phaseoleae.

World production. Discrepancies will be encountered when comparing statistical figures from different sources. Available data from the United Nations (7) show the world production of these five groups of seeds combined* to be 23.8 million metric tons in 1955. There was a

* Excluding soybeans.

steady but slight increase in production in the period 1948–55, the difference between extremes amounting to only 2.7 million metric tons. Figures for 1956 for chick peas and beans show a decrease in production of 0.5 and 0.2 million metric tons, respectively (7).

Vicieae groups. The production of chick peas in 1955 was 6.7 million metric tons, or around 28% of the total legume seed produced; peas amounted to 4.8 million metric tons; broad beans approached a figure of 4.4 million metric tons; and the lentils proved to be the smallest crop among these four, with a world production of 0.6 million metric tons.

*Phaseolus group.** The highly diversified group of species and varieties of common edible beans are the largest of the five groups; 7.3 million metric tons were produced in 1955.

Aside from these five major groups, a number of peas and beans for which no statistical figures are available bear definite technical importance. Some of them will be included in the discussion.

1. The *Vicieae*

a. *Chick Peas*

Whereas the production of chick peas is large, its world distribution is restricted. India and Pakistan produce and consume around 90% of the world production. The rest of the seed produced is absorbed by several Mediterranean countries and to some extent by some of the Latin American republics. Turkey, Ethiopia, French Morocco, and Algeria are relatively important producers. Spain is noted for the traditional use of chick peas in its basic diet. In the American continent, Mexico has for a long time been the leading producer.† A goodly part of the Mexican seed comes from the northern states of Sonora and Sinaloa;‡ most of the production from this area has traditionally been exported, primarily to Spain.

Chick peas are of Caucasian origin and belong to the genus *Cicer*, from which term various names have been derived. *Cece* (Italian) and *pois chiche* (French) are assumed to be corrupted forms of *cicer* (Latin); however, the particular shape of the seed, which resembles the head of a pullet, has given rise to the frequent thought that the

* Does not include a number of other genera within the *Phaseoleae*; accounts for production of *Phaseolus vulgaris*, *P. lunatus*, *P. aureus*, and *P. mungo*.

† From 1946 to 1955 the Mexican production of seed amounted to an average of 98,000 tons per year (8).

‡ Mexico produces two types of chick peas. The large garbanzo from the North constitutes the export crop. The small chick pea (forage seed) grown in the Center accounts for about 60% of the national production.

term "chick" originates from the configuration of the seed. The Spanish-speaking peoples call it *garbanzo*, and the names of Bengal gram (English), *chenata* (Sanskrit), and *chana* (Hindi) are commonly used (9).

The most common varieties of *Cicer arietinum* yield seeds which are usually whitish or slightly brown. Others produce reddish or dark seeds. The chick pea may vary considerably in size and weight.

The chick peas exported from Mexico are mostly classified according to the number of seeds in 28 or 30 g.; the first basis applies to screened seeds, and the latter to unscreened. In the first group one will find the terms "extra," "fine," and "sublime," referring to choice seeds within the limits of 36 to 44 seeds in 28 g. In the second group varying from 40 to 62 seeds in 30 g., nine classifications are commonly employed (fancy, cathedral, select, etc.).



FIG. 1. Chick pea (*Cicer arietinum*) northern variety, dry and swollen in water.

The plant is commonly grown for seed. It bears pods which contain one or two seeds of about 10 mm. in length. The surface of the seed is wrinkled and somewhat spherical except for the protruding radicle and the marginated hilum showing in the longitudinal scar depression between cotyledons (see Figure 1).

b. Peas

The two best-known members are *Pisum sativum*, var. *arvense* Poir., field or smooth pea, and *P. sativum* L., or wrinkled pea.

In 1955, China accounted for production of over 60% of the world crop, or around 3 million metric tons, with India as the second largest producer (589,000 tons). The United States showed a substantial increase in its production between 1955 and 1956, from 127,000 to 233,000 metric tons. In Europe, the Netherlands and the United Kingdom are the leading producers, with a production for 1956 of 77,000 and 71,000 tons respectively. In Africa, the Belgian Congo and Ruanda

Urundi produced 76,000 and 60,000 tons, respectively, in 1955. Argentina is the important producer of South America, with 30,000 tons in 1954 and a decrease to 20,000 tons in 1955.

c. Broad Beans

China (mainland) is the main producer of broad beans, with approximately 3 million metric tons in 1956. In the same year Italy registered a production of 285,000 tons, substantially lower than in 1955 with 464,000 tons. Egypt produced 208,000 tons in 1956. England, Spain, and France are also important producers.

The seed (*Vicia faba*) is also called horse bean and Windsor bean. It is a native of North Africa and the Near East. It is fed to domestic animals and to some extent serves for human consumption.

d. Lentils

India is the largest producer of lentils, with 201,000 tons in 1955. Turkey and Ethiopia come next; each produces over 75,000 tons yearly. Egypt and Syria follow, with 49,000 and 37,000 tons, respectively. Spain, Italy, France, and Greece are the important European producers. The production of Spain amounted to 24,000 tons in 1955-56 (7).

The lentil (*Lens esculenta*) is one of the oldest legumes in cultivation; it is still a common edible seed throughout the world. The Mediterranean area and southwest Asia have been mentioned as its origin, from which it supposedly spread to Western Europe and America.

e. Other Viciae

This tribe includes some other members of the genera *Vicia* and *Lathyrus* of sufficient interest to deserve their being mentioned; some of them are actually bean-shaped. Among the first group are the bitter vetch (*Vicia ervitia*), the spring vetch (*V. sativa*), the winter vetch (*V. villosa*), the narrow leaf vetch (*V. angustifolia*), and the rough hairy vetch (*V. hirsuta*). In the second group is the chickling vetch (*Lathyrus sativus*), which is commonly cultivated in Europe, particularly in the South. Closely related is the sweet pea, a widespread ornamental legume (*L. odoratus*).

2. The Phaseoleae

a. Beans (*Phaseolus*)*

The largest producer of beans is Brazil, with a production in 1955 of about 1.48 million tons of the most common edible types. India comes second, with 1.21 million tons in the same year. The United States produced over 800,000 tons yearly during the period 1948-56.

* Includes *P. vulgaris*, *P. lunatus*, *P. aureus*, and *P. mungo*.

Mexico had a yearly production of around 400,000 tons during 1954-55. Italy is the outstanding producer in Europe, at a level of 140,000 metric tons of dry beans yearly. France and Spain are also important producers, with 120,000 and 96,000 tons of edible beans, respectively for 1956.

Among the various genera attached to the *Phaseoleae*, the *Phaseolus* genus is the most numerous, and the species *Phaseolus vulgaris* is the best known and most widely cultivated. This species is represented by a number of varieties commonly known as kidney beans, field, garden, or haricot beans. In the immature form, there are the green, string, and snap or wax beans. Dry beans most abundantly grown in America include the pinto, black, white, pea bean, red, yellow eye, and great northern. Another *Phaseolus* of interest is *P. lunatus* (including *P. limensis*), which produces the large flat seed called lima or butter bean and which may be found in various forms: sieva, colored, large flat, and potato types. Its probable origin is Guatemala, although Brazil and Peru have at times been considered as sites of origin.

Among the *Phaseolus* species regarded as Asiatic are *P. angularis*, or adzuki bean, *P. mungo*, or urd bean, *P. aureus*, commonly called mung bean, *P. calcaratus*, or rice bean, and *P. aconitifolius*, known as moth bean.

Some well-known American species are *P. acutifolius* and *P. multiflorus*,* known, respectively, as tepary and scarlet runner or multiflora.

The members of the *Phaseoleae* mentioned here have certain common characteristics. Most of them are climbing herbs and only occasionally erect or shrubby plants. A few exceptional species are trees.

b. Other *Phaseoleae*

Several genera within the *Phaseoleae*, such as *Vigna*, *Dolichos*, *Canavalia*, and *Stizolobium*, deserve mention; the so-called cow pea or china bean (*V. sinensis*), the horse gram (*D. biflorus*), the hyacinth bean (*D. lablab*), the black-eye long bean (*D. monochalis*), the jack bean (*C. ensiformis*), and the velvet bean (*S. deeringianum*) are among the better known of this group.

A toxic species of West Africa, *Physostigma venenosum*, calabar or ordeal bean, has been the subject of some interesting studies (10).

3. The Genisteae

a. *Lupinus*

The white (*L. albus*), yellow (*L. luteus*), and blue (*L. angustifolius*) lupines are the most commonly grown members of the *Lupinus* group in America; they are grown especially for soiling purposes. Practically all species are known to contain alkaloids toxic in various degrees to ruminants. Some varieties are, however, non-poisonous (11, 12).

* *P. coccineus* used as well.

IV. COMPOSITION

About seventy of the most common seed-yielding species, including those which are consumed in large quantities by man, are reviewed in this section. The data have originated at different periods and through the application of analytical procedures of varying degrees of accuracy.

Only mature peas and beans are considered in this section. In many areas of the world immature pods are harvested and eaten; the composition and properties of immature seeds have been reviewed by Lee (12a).

1. Components

a. Protein

Protein fractions called vicilin and legumin have been isolated from peas (13). Thirty-four different legume species have been reported to contain similar protein components (14). Legumelin has been considered as a third protein constituent. Phaseolin and concanavalin have been used to designate the main proteins of the common bean and jack bean, respectively; conglutin is the name applied to the main protein fraction of the lupines. (For additional information on vegetable proteins see Chapter 3.)

The total protein content (dry basis) of most legumes described in this chapter falls within the limits of 20 to 30%. A mean value of 26% protein was obtained for a group of 25 varieties of beans (15). The seeds of *Canavalia ensiformis* (jack bean) contain 36% protein. An exceptionally high protein content of up to 50% is found in *L. cruikshanksii* (16).

b. Carbohydrates

Starch is the major carbohydrate of legume seeds. Few members are known to contain little or no starch; in the instances where there is little starch, sugars constitute the largest proportion of the nitrogen-free extract of the seed. The *Lupinus* have been reported as containing no starch and an amount of nitrogen-free extract in the range of 25 to 45% (dry basis). Most other legumes fall within the limits of 55 and 70% nitrogen-free extract.

c. Fat

The majority of the species of legume seeds considered do not contain more than 7% oil, and among these a goodly number contain as little as 1 to 2%. Nine common varieties of beans from Central America

give values for oil content between 0.9 and 4.6%.* The chick peas, *Cicer arietinum*, usually contain 4 to 7% oil (17, 18). The same was found for some members of the *Lupinus* family and for beans of the *Stizolobium* genera (*S. deeringianum*, velvet bean). Some members of *Lupinus* contain somewhat higher levels of from 7 to 10% oil, and a few may even be as high as 16% in oil (16).

d. Crude Fiber

An unusually high content of fiber is encountered in most members of the *Lupinus*, a fact which might constitute a limiting factor when considering them as potential sources of isolated protein. Some *Vicia*, *V. faba* and *V. sativa*, contain 7 and 9.3%, respectively, of crude fiber. The Rangoon bean (*Phaseolus lunatus*) has as high as 7.5% and *Canavalia ensiformis* (jack bean) may contain 7 to 10% crude fiber. The crude fiber content of the group of beans from Central America, previously mentioned,* varied between 3.8 and 5.6%. The most common representatives of the genera, *Lens*, *Cicer*, *Pisum*, *Lathyrus*, *Phaseolus* and *Dolichos*, do not usually exceed 6% crude fiber (16).

e. Vitamins and Minerals

An analysis of vitamins and minerals in several species available in Mexico is given in Table I. Carotene is absent or exists in negligible amounts. Thiamine content varies widely, from 0.12 to 1.09 mg. per 100 g. (dry basis). The beans are for the most part lower in thiamine than are the other related species; values for the others exceed 0.80 mg. per 100 g. Riboflavin content varies less, from 0.10 to 0.34 mg. per 100 g., the highest value corresponding to the broad bean (*V. faba*). Niacin content varies between 1.15 and 2.56 mg. per 100 g. The broad bean, lentils, and peas have a higher content of this vitamin; some varieties of beans contain above 2 mg. per 100 g. of seed. Ascorbic acid is low or absent in all varieties considered (19). The mean values for three of these vitamins in the group of twenty-five varieties of beans, mentioned previously (15), are as follows: niacin 2.44, thiamine 1.11, and riboflavin 0.20 mg. per 100 g. of dry seed. These values correspond to the highest figures shown in Table I.

Examination of Table I shows that the calcium level is highest in the beans. A consistently high content of phosphorous is found in all legumes, the range limits being 241 to 567 mg. per 100 g. The iron content varies between 4.56 and 10.38 mg. per 100 g. of seed.

* N. S. Scrimshaw, Instituto de Nutrición de Centro América y Panamá, unpublished data, 1956.

TABLE I
VITAMINS AND MINERALS IN DRY PEAS AND BEANS FROM MEXICO^a

Species	Caro- tene	Thi- amine	Ribo- flavin	Niacin	As- corbic acid	Cal- cium	Phos- phorus	Iron
mg./100 g. whole seed (dry basis)								
<i>Cicer arietinum</i>								
Chick pea (garbanzo breve) ^b	0.07	0.81	0.19	1.68	0.72	115	438	9.67
<i>Pisum sativum</i>								
Wrinkled pea (alverjón)	0.02	1.01	0.20	2.56	0.00	80	320	8.35
<i>Vicia faba</i>								
Broad bean (haba)	0.09	1.00	0.34	2.52	5.38	54	482	8.01
<i>Lens esculenta</i>								
Lentils (lenteja)	0.08	0.77	0.22	2.20	3.99	82	354	8.26
<i>Phaseolus vulgaris</i>								
White bean (small aluvia)	0.01	0.12	0.20	2.01	3.42	173	448	7.28
White bean (large aluvia)	0.06	0.91	0.23	2.42	3.39	122	409	7.45
White bean (blanco)	0.00	0.70	0.17	1.12	0.00	215	523	5.34
Yellow bean (amarillo)	0.00	0.67	0.13	2.22	0.00	375	517	5.14
Frijol azufrado	0.00	0.55	0.15	1.33	0.00	267	507	5.57
Frijol bayo gordo ^c	0.00	0.76	0.16	1.89	0.00	216	396	7.63
Frijol canelo	0.00	0.62	0.17	1.27	0.00	295	412	4.95
Frijol cócona ^b	0.01	1.09	0.18	1.68	2.61	201	392	10.38
Frijol garbancillo	0.00	0.58	0.15	1.80	0.00	320	567	5.26
Mexican bean (mexicano)	0.00	0.46	0.21	1.15	0.00	299	241	4.56
Black bean (negro) ^d	0.02	0.71	0.19	2.08	1.53	207	417	7.25
Frijol ojo de liebre	0.00	0.78	0.14	1.62	0.00	334	467	5.65
Frijol palacio	0.03	0.95	0.15	1.76	2.35	178	413	7.72
Frijol rosita	0.00	0.61	0.10	1.15	0.00	272	553	4.70

^a R. O. Cravioto, G. Massieu H., and J. Guzmán G., *Ciencia (Mex.)* **11** (5/6), 129 (1951).

^b Mean value for two different commercial sources.

^c Mean value for three different commercial sources.

^d Mean value for four different commercial sources.

See also Table II, page 220, for more data on composition of legumes.

2. Analysis of Components

a. Protein

The amino acid content of crude protein of several legume seeds is shown in Table II (20–23). It is evident that most legumes are good sources of lysine but are somewhat deficient in methionine. The percentage of total essential amino acids in these seeds varied from 8.4 to 10.6% (dry basis).

TABLE II
ESSENTIAL AMINO ACID CONTENT OF PROTEIN OF PEAS AND BEANS

	Broad bean				Chickling vetch				Common beans							
	Garbanzo (<i>Cicer arietinum</i>)	Common pea (<i>Pisum sativum</i>)	(<i>Vicia faba</i>)	Lentils (<i>Lens esculenta</i>)	Kidney (<i>Phaseolus vulgaris</i>)	Lima (<i>P. lunatus</i>)	Urd (<i>P. mungo</i>)	Adzuki (<i>P. radiatus</i>)								
References	(20)	(a, b)	(21)	(20)	(23)	(21)	(15)	(21)	(23)							
Protein ^c content, % (dry basis)	23.3	23.5	23.3	25.1	—	21.0	27.1	27.3	23.0	26.8	25.0	23.8	25.2	23.5	22.0	17.0
Amino acids	g. per 16 g. of nitrogen															
Isoleucine	6.2	5.9	5.1	7.1	5.5	7.1	7.0	6.4	6.5	4.7	8.7	5.5	—	6.0	7.3	9.8
Leucine	14.8	8.9	7.4	10.2	7.0	9.5	9.8	13.1	8.5	7.7	9.3	9.2	—	8.3	10.2	12.3
Lysine	5.7	6.5	6.3	6.3	6.5	8.9	5.7	5.3	7.4	5.8	7.4	7.2	8.2	5.9	7.3	9.2
Methionine	1.0	1.5	1.7	0.3	1.3	0.8	0.3	0.4	0.5	0.8	0.6	1.2	1.0	1.5	1.5	1.3
Phenylalanine	5.0	6.5	6.8	5.2	5.0	4.6	4.9	4.2	3.7	4.6	4.2	6.5	—	6.2	5.3	5.7
Threonine	3.2	4.9	3.7	3.7	3.9	4.2	3.9	3.1	3.9	3.8	4.7	4.5	—	4.7	3.4	4.1
Tryptophan	1.5	1.0	0.7	1.2	0.8	0.3	1.0	1.0	0.3	0.8	0.3	1.1	0.7	0.9	0.4	0.5
Valine	4.5	6.2	6.3	4.7	5.5	6.5	4.9	5.2	5.7	6.4	7.0	7.1	—	7.8	6.9	8.5
Arginine	8.2	6.9	9.0	9.4	7.0	13.4	7.9	7.6	8.5	9.3	12.5	5.3	—	6.1	7.4	10.2
Histidine	2.1	3.0	2.6	2.5	2.2	2.1	1.8	2.0	1.7	2.5	2.1	3.1	—	3.4	2.0	2.5

^a Microbiological determination based on protein extract at pH 7; includes soluble and dispersible fractions.

^b E. Leuze C., of the Instituto Mexicano de Investigaciones Tecnológicas, unpublished work performed at the University of Wisconsin, 1952.

^c Nitrogen $\times 6.25$.

b. Starch

Starch from different plant sources is likely to show variations in physical-chemical properties. The ratio of amylose (straight-chain molecule) to amylopectin (branched-chain molecule) generally approaches 1 to 3. There are some exceptions. Amylose-free starch may, for instance, be obtained from sorghum and from waxy corn. Amylopectin-free starches, however, are not known to exist. Plant materials containing starch with a higher-than-normal amylose content are available, and these may constitute a starting point for the development of plants containing starch with little or no amylopectin. The starch derived from chick peas, Alderman peas, and Steadfast peas contains 33, 65, and 67% amylose, respectively (24, 25).

c. Oil

Legume seed fats are known to contain a large proportion of unsaturated fatty acids in their glycerides. Eicosenoic acid is present in small amounts in some *Leguminosae*. Comparative data on the composition of the lipid fraction of common peas and beans is given in Table III (17, 18, 26–31).

V. TOXIC COMPONENTS OF CERTAIN LEGUME SEEDS

The better-known toxic effects of some legumes may be assigned to the presence of some obviously poisonous substance, such as the alkaloids of the *Lupinus* or of the calabar bean (*Physostigma venenosum*). In these instances well-defined pathological conditions result after ingestion of the causative agent.

Aside from these, there exists a group of diseases of a more complex nature, which arise from eating some peas and beans. In this group of diseases, the corresponding etiology has often been difficult to define, and the interpretation of symptoms has frequently given rise to some confusion. These diseases have generally been referred to as lathyrism, cicerism, or odoratism. The toxic principle or principles involved have been the object of numerous studies.

A well-defined chemical compound, β -(N- γ -L-glutamyl)-aminopropionitrile, has been isolated from the seeds of *L. odoratus* and from singletary peas (*L. pusillus*), the toxicity being assigned to the β -aminopropionitrile portion of the molecule (32–34). Other *Lathyrus* species are likely to contain this compound in various concentrations, as has been demonstrated for *L. strictus* and *L. hirsutus* (35–37). The seeds of *Lathyrus sylvestris wagnerii*, *L. sphaericus*, and *L. tingitanus* are more toxic than the other species mentioned, but this toxicity is not

attributed to β -aminopropionitrile. Toxic effects are convulsion followed by death in several days.

Some of the pathological conditions observed either in human beings or in animals, and in many instances in both, pertain to skeletal lesions, medically regarded as osteochondritis, scoliosis, and epiphyseal lysis with associated necrosis of the femoral head. In many instances malformation of the long bones, spinal and sternal curvature, and enlargement of the metaphyseal and epiphyseal junctions have been observed. In most cases, there is interference with the calcification process in young animals, spasticity and rigidity of the leg muscles, retardation in the sexual development, and many other disorders, frequently found in the bladder, rectum, and genital organs. Various degrees of paralysis are associated with this disease (38–46).

During certain periods, the disease has stricken relatively large groups of people, apparently in the form of an epidemic. Several such instances have occurred in India, North Africa, and Spain, mostly among low-income groups and particularly during times of famine. Between 1945 and 1946 a goodly number of cases of paralysis were observed in Spain; the great majority developed in individuals between the ages of 20 and 30 years. In some instances species of the genus *Cicer* have also been presumably pointed out as the causative agents. In the United States (Wisconsin and Michigan) the disease has been occasionally observed in cattle after the ingestion of seeds of several species of *Lathyrus* (49).

Aside from the β -aminopropionitrile compound, aminoacetonitrile has been shown to produce extensive skeletal lesions similar to those which appear in rats after being fed with *L. odoratus* seeds. The same may be said for bis(β -cyanoethyl)-amine, although in this particular case the behavior of the rats suggests possible cerebellar effects in addition to typical skeletal lesions (47–52).

It is of interest to mention a number of closely related studies which may or may not have a direct bearing on the problem of lathyrism. In some of these studies selenium and manganese have been suggested as the responsible agents of diseases giving rather similar symptoms. It is known that these metals may reach abnormally high levels in certain legume seeds (53, 54). In other instances, the particular amino acid deficiencies of some legumes have been regarded as possible causes (55, 56). (See also Chapter 9 for mention of favism, another type of injury attributed to eating certain legumes.)

The aminonitrile compounds so far studied are readily soluble in water and may be extracted from the seeds by soaking. The common practice in preparing dishes containing some of these legumes is first to soak the seeds and to throw away the steeping water. This practice

may prove, in the long run, useful in diminishing the risks derived from their ingestion.

VI. USES

A discussion on uses of peas and beans as human food has been presented in Chapter 9. Dry peas and beans are almost exclusively used in foods and feeds; they are not processed further to concentrate their constituents. Some species, as the broad bean and the chickling vetch, find use as foodstuffs for both human beings and animals. Others, such as the *Lupinus*, are restricted to fodder. The seed of most members is edible.

The greatest proportion of the seed is consumed directly in the home, and a diversification of dishes may be found all over the world with one seed or another of this group in the role of major ingredient. At times some of these dishes constitute the most popular culinary confection of a country. A group of classical recipes have become internationally known. Some of them have been carried to an industrial level, as canned, dehydrated, baked, and roasted products of commerce. Some efforts have been made to produce presoftened beans, which may be cooked in a much shorter period of time (57). A few species are utilized as partial substitutes and adulterants for coffee. The ground seeds (meals) of a number of species such as kudzu, lespedeza, and cow peas are currently used in feed mixes.

VII. POTENTIAL USES

There are a number of promising applications for peas and beans, especially in the field of food enrichment, that have not as yet been developed to any practical extent. The ever-increasing knowledge of limitations of our present food habits and the recognition of the need for their improvement, however, are changing traditional outlooks on the subject. Already discussed in previous chapters are the possibilities of new uses for oilseed protein isolates, particularly soybean protein. (See Chapters 10, 11, and 15.) We shall illustrate similar potential applications to peas and beans by describing some of the results of research on chick peas.* A number of products derived from these seeds are of interest as possible ingredients for enrichment. Among these are flour, undefatted protein, and purified protein.

1. Scope of the Problem

In a number of countries, large sections of the population depend on a basic diet which is low in animal protein; the protein of their

* Unpublished data from research carried out by the writer and co-workers at Instituto Mexicano de Investigaciones Tecnológicas (1951-57), Mexico City.

diet is mostly derived from vegetable sources. In these instances substantial improvement may be achieved through the proper combination of vegetable proteins of different amino acid compositions.

In some of the Latin American countries, for instance, the basic diet of low-income groups is principally composed of corn products derived from a conventional wet corn dough prepared by steeping the grain in hot lime water. The various types of food products made from it are certainly lacking in ingredients which are important in nutrition. For example, lysine and tryptophan are amino acids which are partially lacking in the daily diet of consumers of these products.

The development of modern processes for the production of a dry stable corn flour* now operating, for instance, in Mexico† has led to a number of studies where soybean meal, chick pea flour, chick pea protein products, soybean proteins, and other promising ingredients have been considered for use in the improvement of the corn meal.

2. Effects of Supplementation

In order to visualize in a preliminary manner the nutritional implications of the addition of chick pea flour and chick pea protein to wheat flour or to corn flour, the amount of essential amino acids present in the original flours and in the enriched products compared with the daily amino acid requirements for human adults are given in Table IV.

Supplementation increases the protein content of the flours, particularly when protein isolate is added. This in itself is a significant accomplishment since, as pointed out by Dean (Chapter 9), the major problem in protein malnutrition is to increase the quantity of protein eaten. But this type of supplementation also improves the quality of the protein. The lysine and tryptophan contents of the mixed flours are increased; the supplemented flours come nearer to satisfying the amino acid requirements of adult humans. There is, of course, the question of the availability of the amino acids in these flours which could influence the nutritional value of the mixtures.

It may turn out that the supplemented flours are inadequate in methionine content. Further supplementation with cottonseed or sesame flours or protein isolates could provide an even more balanced protein diet. (See Chapter 13 for a discussion of the problem of supplementation of cereals with oilseed meals and with synthetic lysine and methionine.)

* Flash-dried product derived from lime treatment.

† A 300-ton plant has been erected in the neighborhood of Mexico City based on a process developed at Instituto Mexicano de Investigaciones Tecnológicas. A similar plant based on the same process is operating in Sherman, Texas.

TABLE IV
EFFECT ON AMINO ACID COMPOSITION OF WHEAT AND CORN FLOURS BY ADDITION OF CHICK PEA PRODUCTS AND SOYBEAN MEAL

Source of protein	Protein ^a (dry basis)	Amount containing 100 g. protein (dry basis)	Amounts of amino acids in 100 g. of protein							
			Leucine	Methio- nine	Phenyl- alanine	Lysine	Valine	Iso- leucine	Threo- nine	Trypto- phan
	%	g.	g.	g.	g.	g.	g.	g.	g.	g.
Flours										
Typical wheat flour ^b	12.5	800	7.0	2.0	5.5	1.9	4.1	4.2	2.7	0.8
Whole corn flour ^b	9.6	1042	15.0	3.1	5.0	2.3	5.3	6.4	3.7	0.6
Additives										
Soybean meal ^b	45.0	222	8.0	1.7	5.3	6.8	5.3	6.0	3.9	1.4
Whole chick pea flour ^c	23.5	426	8.9	1.5	6.5	6.5	6.2	5.9	4.9	1.0
Undefatted chick pea protein ^c	60.0	167								
Chick pea protein powder ^c	90.0	111								
Enriched wheat flour products										
20% Soybean meal	19.0	526	7.5	1.9	5.4	4.2	4.7	5.1	3.3	1.1
10% Chick pea flour	13.6	735	7.3	1.9	5.7	2.7	4.5	4.5	3.1	0.8
50% Chick pea flour	18.0	556	8.2	1.7	6.2	4.9	5.5	5.3	4.1	0.9
10% Undefatted chick pea protein	17.3	578	7.6	1.8	5.8	3.5	4.8	4.8	3.5	0.9
10% Chick pea protein powder	20.3	493	7.8	1.8	5.9	3.9	5.0	4.9	3.7	0.9
Enriched corn flour products										
20% Soybean meal	16.7	599	11.2	2.3	5.2	4.7	5.3	6.2	3.8	1.0
10% Chick pea flour	11.0	909	13.7	2.8	5.3	3.2	5.5	6.3	3.9	0.7
50% Chick pea flour	16.6	602	10.6	2.0	6.0	5.3	5.9	6.0	4.2	0.9
10% Undefatted chick pea protein	14.6	685	12.5	2.5	5.6	4.0	5.7	6.2	4.2	0.8
10% Chick pea protein powder	17.6	568	11.9	2.3	5.8	4.5	5.8	6.2	4.3	0.8
Essential amino acid requirements per day (adult) ^d			2.2	2.2	2.2	1.6	1.6	1.4	1.0	0.5

^a Nitrogen X 6.25.

^b R. Block and D. Bolling, "The Amino Acid Composition of Proteins and Foods," 2nd ed. Charles C. Thomas, Springfield, Illinois, 1951.

^c E. Leuze C., of the Instituto Mexicano de Investigaciones Tecnológicas, unpublished work performed at the University of Wisconsin, 1952.

^d Based on the recommended safe daily intake which is twice the minimal amount listed in Table III, Chapter 2, and in Table VIII, Chapter 18.

3. Additives

a. Chick Pea Flour

The use of chick pea flour as an additive for enrichment of foodstuffs presents certain limitations. It is, however, the cheapest of the products derived from the seed and may be used advantageously in a wide group of food products. This should hold true similarly for flours from other legume sources.

Chick pea flour can be used in the enrichment of a number of bakery products up to a certain amount only, for otherwise the baking properties of the mixture are increasingly affected. This may be explained mostly in terms of the relative decrease in wheat gluten concentration of the final mixture.

When chick pea flour is used up to 10% level with wheat flour, the organoleptic properties of the mix do not differ appreciably from those of wheat flour itself. The enriched product behaves in much the same manner as do ordinary wheat preparations. Baking tests show that the water absorption, mixing requirements, and general mechanical tolerances are not substantially modified. Dough consistency is slightly lower than for 100% wheat flour. Tests with the extensograph show a slight increase in extensibility of the dough and a minor decrease in resistance to extension and volume potentialities during fermentation.

The experimental preparation of chick pea flour through the use of dry milling equipment (Brabender type) gives the following products:

Yields of flour obtained in a single grinding are: grade A, 73%; grade B, 22.2%; residue, 4.1%. Yields of products obtained in two-step grinding are as follows: grade A, 82%; grade B, 13%; bran, 4%. These products show a protein content of 25.4% and 22.6%, respectively. The bran contains 15.3% protein.

b. Chick Pea Protein Products

Some of the limitations which are likely to apply when the whole seed or flours are used are evidently eliminated with the substitution of highly concentrated proteinaceous materials. The effect on composition which may be expected as a result of the addition of these materials to foodstuffs is also given in Table IV.

The addition of chick pea protein to corn flour at the level of 5% does not modify to any appreciable extent the organoleptic characteristics of the enriched product. Supplementation at a 10% protein level may be detectable, although the pleasant flavor which results has been considered as probably not objectionable. The actual amount which may be added depends to a great extent on the degree of purification of the material and on the price of the final product.

A number of technical problems will have to be solved before an economical protein isolate of this type can be made available. One approach to the preparation of highly concentrated proteinaceous materials from these sources is the use of wet milling processes. It has been possible by such means to obtain protein from chick pea of a varying degree of purity depending on the processing conditions.

An undefatted protein containing 25 to 30% oil requires a more simplified process than preparation of oil-free proteins. A typical analysis of undefatted protein obtained at the pilot-plant level is as follows: protein, 60%; oil 28%; mineral matter, 3%; and nitrogen-free extract, 9%. The use of undefatted protein is conceivable for a number of applications where the oil does not constitute a disadvantage. It will, however, require the use of suitable antioxidants in order to improve its keeping qualities. A high-quality starch is obtained along with the protein as a coproduct. The yields obtained from the seed (dry basis) are: undefatted protein, 25%; starch, 38%; and cattle feed, 15%. Under these conditions a 22% loss is accounted for as sugars, low-molecular weight protein fractions, salts, and other minor constituents.

Highly purified, oil-free chick pea protein may be obtained by means of a more elaborate process. Since the oil of the seed is distributed throughout the cotyledons, its separation by mechanical means as in the cereal grains is not possible. The protein acts as a carrier of the oil throughout the wet process and will be obtained as an undefatted product, unless means for the extraction of the oil are introduced prior to the final concentration and drying of the protein. The solvent extraction of the seed as a whole, as a first step of the process, has been found objectionable because of resulting undesirable modifications in the starch and protein. The purified protein obtained by use of counter-current extraction applied to the wet protein stream has the following characteristics (dry basis): protein content, 95%; residual oil, 4%; impurities, 1%. The amount of oil in the isolated product may be lowered further by the introduction of a more efficient extraction system.

It is of interest to mention that the dispersibility of such isolated protein in water at pH 10.5 is approximately 99.5%.

The purified protein may also be obtained from the dry undefatted protein product by direct solvent extraction of the flaked or pelleted material. The purity depends to some extent on the solvent; when common petroleum solvents are used, the degree of purity is much lower than when ethyl and methyl alcohols are the solvents. The dispersibility of protein made by this procedure is only about 60%, indicating that there has been denaturation.

Isolation of purified chick pea protein from the whole seed gives

the following estimated yields (dry basis): purified protein, 17.5%; starch, 38%; oil, 5.5%; and cattle feed, 15%, with an over-all recovery of 76% of the original raw material.

The purpose of the discussion in this section has been to explore the possibilities of new approaches to improving legumes as human food. Isolated legume protein seems to have advantages in supplementation of cereals over that of legume seeds or of flour from the seed. Whether this can provide a sound and economic solution to the problem of improving the protein nutrition of many populations is a challenge to modern technology.

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CHAPTER 27

FERMENTATION FEEDSTUFFS

C. S. BORUFF AND J. M. VAN LANEN

I. INTRODUCTION

Vegetable protein materials considered under the title of this chapter are animal feed ingredients obtained as products or by-products of fermentation. They are largely but not exclusively of vegetable origin. In general, fermentation feedstuffs are comprised of the non-fermentable portion of fermentation substrates combined with the microbial cells and secondary metabolites of the particular organism used that are not otherwise recovered. Exceptions are brewers grains and malt sprouts obtained in brewing and malting, respectively, which are separated prior to fermentation.

The main items produced in quantity are by-products from the production of beverage and industrial alcohol, beer, and industrial solvents such as acetone and butanol. In each process, carbohydrate is fermented directly or separated for subsequent fermentation, thus enriching the residues in protein. In addition, microbial protein and other nutrients are synthesized by the fermenting organism so that feed by-products are generally enhanced in nutritional value beyond mere concentration of the non-fermentables. Besides the fermentation by-products produced in larger tonnage, many fermentation products containing vitamin B₁₂, riboflavin, antibiotics, and unidentified growth factors are being produced in increasing amounts as feed ingredients. These are incorporated into feedstuffs at relatively low levels to meet specific nutritional needs, and thus they contribute little to the protein requirements of the animal.

The feeding of fermentation residues to animals is an old practice which has been carried out in various parts of the world for many years. Initially these residues were fed in wet form, and, because of their limited storage life, usage was necessarily restricted to local areas surrounding fermentation plants. Beginning about 1930 various improvements in recovery equipment began to be developed in the United States so that today a high percentage of fermentation residues are re-

covered in dry form. During and since World War II, industrial fermentations have increased in volume and versatility. Feed by-products from these various processes have come to play an important part not only in fermentation technology and economics but also in animal feed production and in agriculture in general.

II. GRAIN DISTILLERS* FEEDS

1. General Information

The principal grains processed by the distilling industry are corn, rye, and barley malt. Other grains and grain products used in small amounts include grain sorghums, wheat and wheat milled products,

TABLE I
UNITED STATES PRODUCTION OF DISTILLERS FEEDS^a

Year ending Sept. 30	Distillers dried grains	Distillers dried grains with solubles	Distillers dried solubles	Total
short tons				
1936	240,400		0	240,400
1939	148,600		500	149,100
1942	341,700		3,700	345,400
1945	352,600	195,400	85,500	633,500
1948	130,100	147,800	73,200	351,100
1951	199,700	288,600	145,600	633,900
1955	66,300	130,900	53,000	250,200
1956	67,600	166,200	51,800	285,600

^a Source: Grain Division, Agricultural Marketing Service, U.S. Department of Agriculture.

barley, and rice. Annual Reports of the Internal Revenue Service show that the industry uses about 70% corn, 15% rye, 11% barley malt, and 4% of various other grains and grain derivatives. During periods of abnormal operation when these preferred raw materials are not available, the industry produces alcohol from any of a number of starch or saccharine materials; feed by-products will vary to some extent accordingly. Grain distilleries normally process less than 1% of the total United States grain crop and return to the farm about one-third of the weight of grain processed as distillers feeds.

2. Production (United States)

Table I summarizes the United States production of distillers feeds from 1936 through 1956. Prior to 1944, distillers dried grains and dis-

* This is the convention adopted for this Chapter. The spelling "distillers'" and "brewers'" is also used widely.

tillers dried grains with solubles were not reported separately. Production of distillers dried solubles was begun in 1939 and reached substantial proportions by 1945. The total annual production of grain distillers feeds ranged from a low of 185,700 tons in 1953 to a high of 633,500 tons in 1945. Anticipated production by the industry should yield from 250,000 to 300,000 tons of feedstuffs annually.

3. Process

A flow diagram of the distilling and feed recovery process is shown in Fig. 1. Cereal grains are ground, slurried with water, cooked batch-

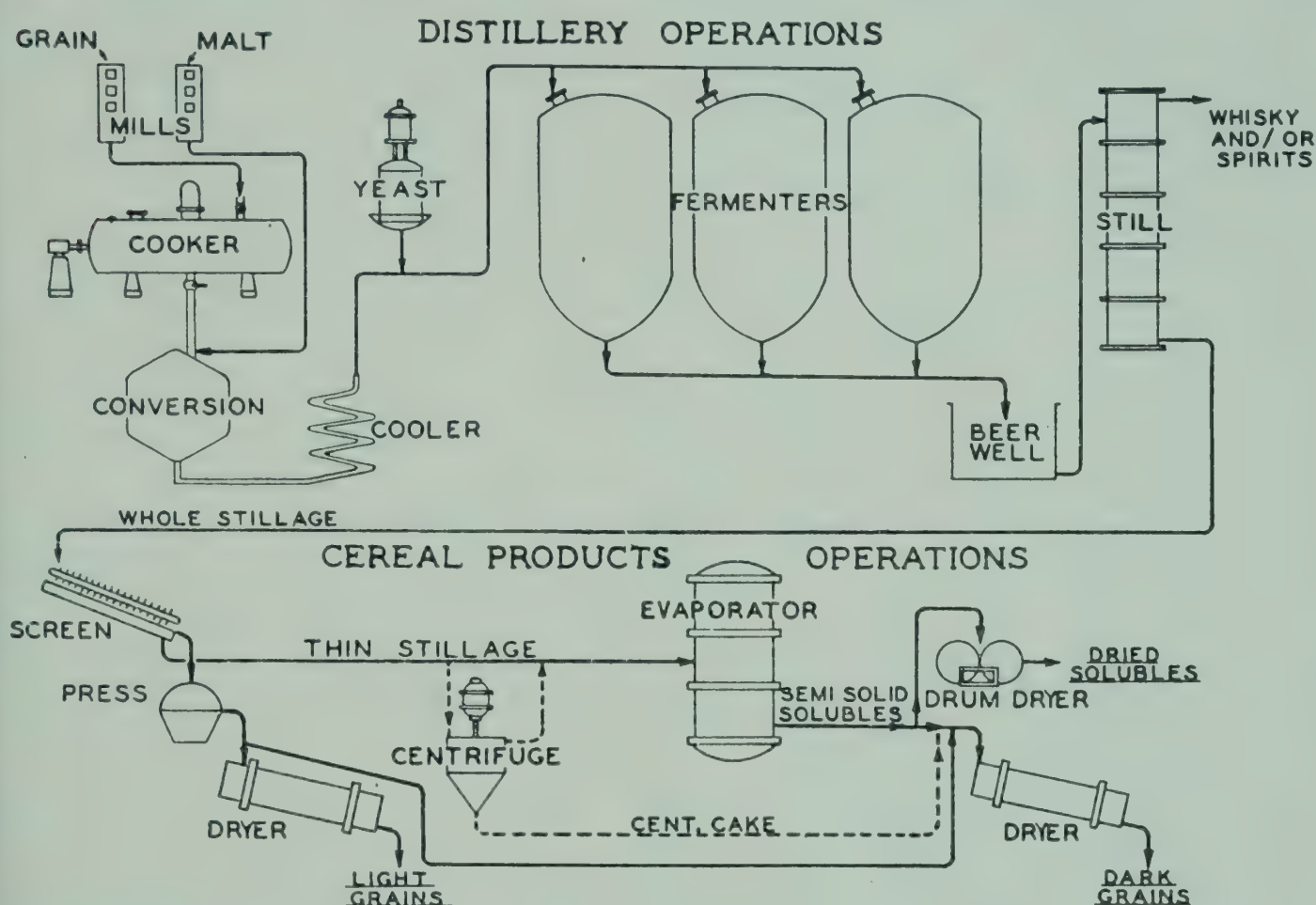


FIG. 1. Flow diagram of grain distilling and by-product recovery.

wise or continuously, cooled and saccharified with ground malt. Fungal amylase also may be used as a saccharifying agent to produce grain spirits (1). After a holding period during which saccharification occurs, the mash is cooled to fermentation temperature, inoculated with yeast, and fermented. After three to four days, the fermented mash or beer is distilled to recover the alcohol or the whiskey distillate. The still bottoms (stillage) serve as the raw material for the recovery of distillers feeds.

Whole stillage containing from 5 to 10% solids consists of spent grains and minerals combined with yeast and yeast products formed during the fermentation. The coarse suspended solids of whole stillage are recovered by a three-step process of screening, dewatering, and drying. This product, generally dried in a rotary steam tube dryer, is

known as distillers dried grains or light grains. Screened or "thin stillage" is concentrated to a syrup containing 25 to 35% solids in a multiple-effect evaporator and dried on a drum (or spray) dryer to produce a product known as distillers dried solubles. In one modification of the recovery, the fine suspended solids or screened stillage are

TABLE II
ANALYSES OF GRAIN DISTILLERS FEEDS^a

Constituent	Distillers dried solubles	Distillers dried grains	Distillers dried grains with solubles
	<u>%</u>	<u>%</u>	<u>%</u>
Moisture	5-6	10-11	10-11
Protein (N × 6.25)	25-30	26-27	26-27
Fat	6-12	8- 9	8- 9
Fiber	3- 4	11-13	8- 9
Ash	6-12	2- 3	4- 6
Calcium	1.4	0.1	0.4
Phosphorus	1.7	0.4	1.0
TDN	77	82	81
Vitamins	<u>mg./lb.</u>	<u>mg./lb.</u>	<u>mg./lb.</u>
Thiamine	4	0.5	2
Riboflavin	8	2.0	4
Niacin	50	21	30
Pantothenic acid	7	2	4
Choline	2500	600	1000
Biotin	1	—	—
Pyridoxine	4	—	—
<i>p</i> -Aminobenzoic acid	4.5	—	—
Folic acid	0.5	—	—

^a Taken from L. E. Carpenter, *Feedstuffs* **27** (9), 30 (1955).

Amended to include total digestible nutrients (TDN) and a range of analytical values for different grains and recovery methods used.

separated by centrifugation prior to evaporation and combined with the coarse grains fraction (2). Distillers dried grains with solubles (dark grains) is made by mixing evaporated thin stillage with screened grains and drying in a rotary tube dryer. By definition (3) this product must contain not less than three-fourths of the solids of whole stillage. Further details on the recovery of grain distillers feed by-products are given by Cooley (4), process flow diagram (5), and by Carpenter (6).

4. Composition

Table II presents typical analyses of grain distillers feeds. (A publication by the National Research Council (6a) contains additional data

on distillers feeds and other fermentation by-products.) Fermentation of the carbohydrate fraction of the grains mashed results in about a threefold enrichment of protein and fat in the feed by-products. The "solubles" fraction is characteristically lower in fiber and higher in water-soluble minerals and vitamins than the "grains." In addition to the vitamins listed in Table II, distillers dried solubles contains approximately 3600 mg. of inositol and 1.14 mg. of β -carotene per pound.

TABLE III

ESSENTIAL AMINO ACID COMPOSITION OF GRAIN DISTILLERS FEED BY-PRODUCTS

Amino acid	Distillers dried solubles			Distillers dried grains			Distillers dried grains with solubles		
	In material (%)	In crude protein ^a (%)		In material (%)	In crude protein ^a (%)		In material (%)	In crude protein ^a (%)	
Arginine	0.6-1.4 ^b	1.2 ^c	4.3 ^c	0.9-1.2 ^b	1.1 ^c	4.2 ^c	0.9-1.1 ^b	1.1 ^c	4.0 ^c
Histidine	0.6-0.8	0.7	2.4	0.6-0.9	0.6	2.4	0.8-0.9	0.8	2.7
Isoleucine	1.1-1.7	1.1	4.1	1.6-2.2	0.9	3.6	2.0-2.5	1.2	4.2
Leucine	1.4-3.3	2.4	8.7	1.8-2.5	2.3	8.9	1.4-2.4	3.0	10.6
Lysine	0.7-1.5	0.9	3.3	0.8	0.8	3.1	0.7-0.9	0.8	2.7
Methionine	0.4-0.5	0.6	2.0	0.4	0.5	1.9	0.5	0.6	2.2
Phenylalanine	1.2-1.7	1.3	4.6	1.1-1.8	1.0	4.0	2.1-2.2	1.3	4.5
Threonine	0.9-1.2	1.1	3.9	0.7-1.0	0.9	3.4	1.1-1.2	1.1	3.9
Tryptophan	0.1-0.2	0.3	0.9	0.2	0.2	0.8	0.1	0.2	0.8
Valine	1.4-1.9	1.5	5.3	1.2-1.9	1.2	4.6	1.8-2.2	1.5	5.3
Crude protein (N \times 6.25)		27.7	100		25.8	100		27.8	100

^a Calculated as grams of amino acid in 16 g. of sample nitrogen.

^b Taken from H. H. Williams, *Feedstuffs* **23** (6), 18 (1951), and C. S. Boruff, *Ind. Eng. Chem.* **39**, 601 (1947).

^c Taken from C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food. Chem.* **4**, 1008 (1956). Data obtained on a composite sample prepared in 1955.

The amino acid content of a number of distillers feed products is shown in Table III (7-9). Baumgarten *et al.* (10) observed that the amino acid composition of distillers feeds is largely a reflection of the grains processed and can be predicted quite accurately from an analysis of the mash bill. Although differences between actual and calculated values were found in this study to be not significant, yeast protein is synthesized during fermentation (11) and does effect some modification of the protein present in the original grains. Spanyer and Thomas

(11a) have attempted to relate the amino acids of yeast and distillers solubles. When the data are adjusted to equivalent nitrogen contents, Baumgarten *et al.* (10) found the amino acid composition of "grains" and "solubles" to be essentially the same.

5. Uses in Feeding

a. Distillers Dried Grains

Distillers dried grains, both light and dark, are well-accepted ruminant feedstuffs. These products have bulkiness, a pleasant odor, and excellent palatability. Although somewhat lower in protein content than oilseed meals, they are relatively high in fat content and especially high in total digestible nutrients. Distillers dried grains find their main use as supplements in dairy rations and, when so used, have been demonstrated to increase milk production and content of milk fat (12, 13). The beneficial effect of distillers dried grains in dairy feeding has been ascribed to their high available energy content, their favorable influence on roughage utilization, and the possible presence of a specific lactogenic factor. Distillers dried grains also are widely used for fattening (14) and wintering (15) beef cattle, for fattening lambs (16), and as a supplement and milk replacement for young dairy calves.* Distillers dried grains may be fed at levels up to 40% of the total ration. Feed formulations containing distillers dried grains have been summarized in a DFRC bulletin (16).

b. Distillers Dried Solubles

Distillers dried solubles is a medium-brown, flaked material having a pleasant odor and moderate acid taste. This product is low in fiber and intermediate in protein and fat content. It is used principally as a source of water-soluble vitamins and accessory growth factors in chick, turkey, and swine rations (17–22). At least three unidentified chick growth factors, two organic and one inorganic, have been postulated (23–25) to exist in distillers dried solubles. Feeding levels of 1 to 5% are recommended for poultry (26) and 2.5 to 10% for swine (22). There are also indications that this product is suitable in milk replacement formulas for feeding young calves (27, 28).

In addition to its uses in feedstuffs, distillers dried solubles supports good microbial growth and is employed as a component of fermentation media for the production of vitamins, enzymes, and antibiotics (29). It has been studied to a limited extent as a human foodstuff (30). Feed

* S. T. Slack, unpublished data.

formulations containing distillers dried solubles have been summarized in a DFRC bulletin (16a).

6. Storage and Handling

For the most part, distillers dried grains (with and without solubles) are handled in bulk by screw conveyor or airveyor systems. A small percentage is sacked. If properly cooled and adjusted in moisture content to between 7 and 11%, this product can be stored for long periods. If stored in bulk, occasional moving or circulation is recommended to prevent the formation of high-moisture areas with subsequent spoilage. Heating and mold damage occur if the moisture content exceeds 16%, although spontaneous combustion has been reported occasionally with low-moisture products.

Distillers dried solubles, because of its hygroscopic tendency, is packaged in moisture-proof sacks. When uniformly dried to a moisture content of 5% or less and placed in a dry, cool location, this product can be stored for long periods.

7. Trends

Research on the engineering, chemical, and nutritional aspects of distillers by-product feeds has provided the basic information necessary to recover and properly utilize these products in modern feed formulations. Production and demand appear to be reasonably well stabilized.

III. BREWING AND MALTING BY-PRODUCTS

1. General Information

Feed by-products of the brewing and allied malting industries are brewers grains, malt sprouts, dried spent hops, and brewers dried yeast. (For a discussion of brewers yeast see Chapter 29.)

Brewers dried grains are defined (3) as the dried, extracted residue of barley malt, alone or in mixture with other cereal, grain, or grain products resulting from the manufacture of wort. Malt sprouts is the product obtained by the removal of sprouts from malted barley together with malt hulls and other parts of malt (official definition). Dried spent hops is the product obtained by drying the material filtered from hopped wort.

As raw materials, the American brewing industry uses about 65 to 80% barley malt, 20 to 35% adjuncts,* and 1 to 2% hops (31).

* Adjuncts are unmalted cereal grains, grain fractions (largely rice, corn grits, etc.), and sugars and syrups which provide additional sugar and extract.

Lesser amounts of adjuncts are used in Great Britain, and Continental beers generally contain no adjuncts (32). Brewers grains represent that portion of malt and adjuncts not solubilized in mashing.

2. Production

Table IV summarizes production of brewers grains in the United States since 1935. Over the past ten years, production has averaged slightly over 225,000 tons annually. About 60,000 tons of malt sprouts are produced per year. In the United Kingdom, 284,000 tons of brewers

TABLE IV
UNITED STATES PRODUCTION OF BREWERS GRAINS^a

Year ^b	Short tons
1936	98,900
1939	104,000
1942	168,700
1945	217,400
1948	233,000
1951	238,900
1955	238,400
1956	243,500

^a Source: Grain Division, Agricultural Marketing Service, U.S. Department of Agriculture.

^b Year ending September 30.

grains (presumably the sum of wet and dry) were produced in 1951 (33). Total feed by-products from brewing, if fully recovered, represent about one-third of the weight of raw materials processed.

3. Process

Figure 2 is a flow diagram of the brewing process. Clarified wort, which is subjected to fermentation, is prepared by mashing ground malt under time and temperature conditions which provide the desired conversion of starch to dextrins and fermentable sugars. Adjunct, usually cooked separately, is added stepwise to the malt mash. After the conversion end point is reached, the mash is clarified by filtration, centrifugation, or lautering. The wort is then boiled, and hops are added during the boiling. Hops and protein coagulated during the heating are removed, and the cooled wort is fermented. See Hinds (32), Schwarz (34), and Tenney (35) for further details of brewing technology.

Spent brewers grains, separated in a lauter tube, filter, or cen

trifuge contain about 20 to 25% solids. In the larger breweries such grains are dewatered and dried, or dried directly to 8 to 10% moisture content usually in a steam tube dryer. One continuous process for drying brewers grains is in use (36). Malt sprouts are separated from kilned malt by screening and cleaning. Hops, where recovered, are dried with the spent brewers grains. Malt houses and breweries operated in conjunction may dry malt sprouts and hops with brewers

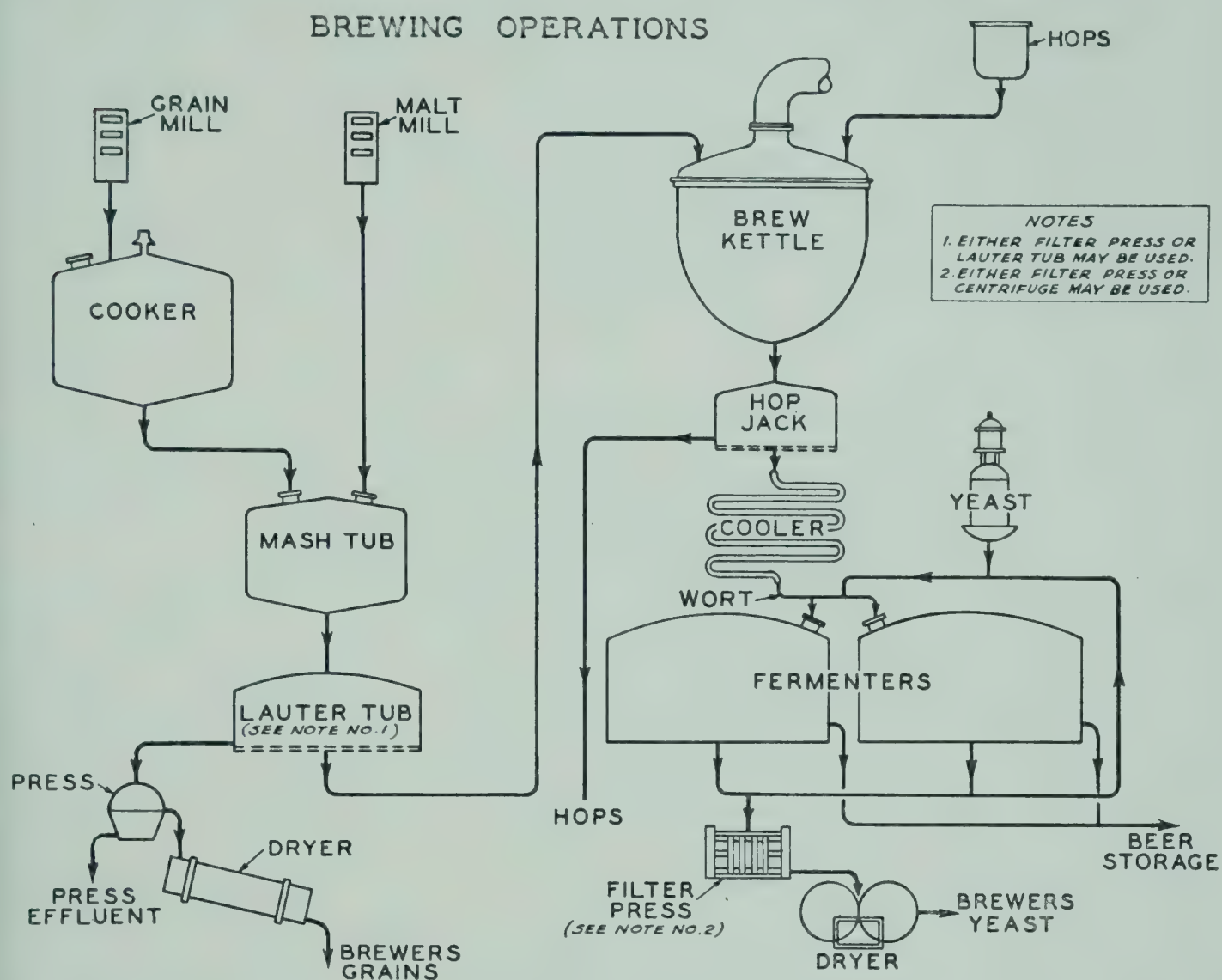


FIG. 2. Flow diagram of brewing operations and by-product recovery.

grains. The screen analysis of brewers grains varies with the mashing process used (as lautering requires a coarser grind than filtering) in the preparation of wort.

4. Composition

Typical analyses of brewers grains, malt sprouts, and spent hops are shown in Table V. It may be seen that malt sprouts are comparable to brewers grains in protein content but are lower in their content of fat. Siefker and Brasch (37) describe a method of mechanically separating the fibrous and proteinaceous fractions of brewers grains to yield a product containing 45% protein. Spent hops are appreciably lower in protein and higher in fiber content than either brewers grains

TABLE V
TYPICAL ANALYSES OF BREWING AND MALTING BY-PRODUCTS

Constituent	Dried brewers grains	Malt sprouts	Spent hops
	<u>%</u>	<u>%</u>	<u>%</u>
Moisture	8	7.4	8
Crude protein (N × 6.25)	25	26.8	17
Fat	6	1.3	4.9
Crude fiber	15	14.2	27.7
Ash	3.8	6.0	5.9
Calcium	0.2		
Phosphorus	0.5		
Vitamins	<u>mg./lb.</u>		
Thiamin	0.3		
Riboflavin	0.5		
Niacin	19		
Pantothenic acid	6		
Choline	600		

TABLE VI
AMINO ACID CONTENT OF BREWING BY-PRODUCTS^a

Amino acid	Brewers grains ^b		Malt sprouts ^c	
	In material %	In crude protein (%) ^d	In material %	In crude protein (%) ^d
Arginine	1.5	5.0	1.1	4.2
Histidine	0.6	2.2	0.5	1.9
Isoleucine	1.4	4.7	1.1	3.9
Leucine	2.8	9.7	1.5	5.5
Lysine	1.0	3.5	1.2	4.5
Methionine	0.6	1.9	0.4	1.3
Phenylalanine	1.7	5.8	0.8	2.9
Threonine	1.1	3.6	0.9	3.4
Tryptophan	0.4	1.3	0.3	1.3
Valine	1.6	5.6	1.4	5.1
Crude protein (N × 6.25)	29.0		27.0	

^a C. M. Lyman, personal communication, 1955.
^b Average of 4 samples.
^c Average of 2 samples.
^d Calculated as grams of amino acid per 16 g. of nitrogen.

or malt sprouts. Amino acid analyses of brewers grains and malt sprouts are shown in Table VI. These products and distillers grains with solubles are generally similar in amino acid composition. The vitamin content of brewing materials, beer, and by-products has been reviewed by Norris (38).

5. Uses in Feeding

a. Brewers Dried Grains

The chief use for this product is in ruminant feeding. Brewers grains are intermediate in protein content but relatively high in fat content and in total digestible nutrients. As such they rank above wheat bran and oats but slightly below corn gluten meal and grain distillers feed by-products. Dairy rations consume the bulk of this product, but it is recommended also for beef cattle, sheep, and horses. For dairy cattle, grain mixtures may contain from 10 to 25% brewers grains, and for beef cattle and sheep about 15% brewers grains. Because of their bulkiness and high fiber content brewers grains are less useful in swine and poultry feeds. Schaible (39) has summarized the applications of brewers feed by-products in animal feeding.

b. Hops and Malt Sprouts

Spent hops are unpalatable when fed alone but can be satisfactorily blended with brewers grains in the proportion in which they are produced and fed to cattle and horses (40).

Malt sprouts may be fed to ruminants at a level of 10 to 15% of grain mixtures. Palatability is reduced with higher levels. Crampton and co-workers (41, 42) have found that feeding from 8 to 16% malt sprouts in swine rations stimulates growth. They concluded that this effect is not attributable either to the biological value of the protein or to the high digestibility of the crude fiber of this product.

6. Storage and Handling

Brewers dried grains are handled in 100-pound sacks and in bulk; conventional equipment for mixing and conveying is used. They can be stored for long periods of time at 6 to 9% moisture content. Grains stored at above 10% moisture content are subject to heating; below 5% moisture there is some danger of dusting and spontaneous combustion.

IV. MOLASSES FERMENTATION BY-PRODUCTS*

1. Molasses Distillers Condensed Solubles and Molasses Distillers Dried Solubles

a. Raw Materials and Methods of Manufacture

The alcoholic fermentation of molasses is carried out in most countries where molasses is available from sugar production; this fermenta-

* This section on molasses fermentation by-products was contributed by Dr. H. J. Prebluda, U.S. Industrial Chemicals Co., Division of National Distillers and Chemical Corp.

tion has been described by Hodge and Hildebrandt (43). The disposal of plant effluents or stillage has always presented an economic problem to industries utilizing molasses as a raw material. In the manufacture of ethyl alcohol from blackstrap molasses by yeast fermentation, the non-fermentable residues range from 6 to 8 pounds per gallon of 190 proof alcohol produced or per 2.4 gallons of molasses fermented (8). High-test molasses has a higher ratio of fermentable sugars and, therefore, yields a smaller amount of residual by-product. The standard methods for making molasses distillers condensed solubles involve efficient multiple-effect vacuum evaporation procedures which are preceded by a preheating process to remove gypsum and coagulated yeast protein. Condensed solubles are evaporated to approximately 45 to 55% solids. The dried product is made by either roller drying or spray drying the condensed material.

b. Production

The production of molasses distillers condensed solubles and molasses distillers dried solubles has varied with the amount of ethyl alcohol produced from blackstrap molasses. With the present trend toward the manufacture of ethanol from petrochemical sources, production of the condensed material in the United States for sale to the trade is not more than 10,000 tons or so per year. During World War II, the fermentation industry sold as much as 250,000 tons of the condensed material per year. The dry product has had the same production irregularities. Prior to World War II, as much as 50,000 tons of molasses distillers dried solubles were produced per year.

c. Composition

The range of analyses for molasses distillers dried solubles products is listed in Table VII. The variation is due primarily to the raw materials and the amount of "backslopping" used in the process. The condensed material would have relatively the same proximate and vitamin analysis on a dry basis as the molasses distillers dried solubles.

The vitamin content of the products of various manufacturers also varies considerably, depending on the process and raw material used.

Table VIII (9) shows the amino acid composition of two molasses distillers dried solubles products differing in protein content.

d. Uses in Feeding

Molasses distillers dried solubles are used successfully in manufactured feeds as a source of unidentified growth factors and vitamins of the B-G complex. The exact amount used in rations depends on the type

TABLE VII

TYPICAL PROXIMATE AND VITAMIN ANALYSES OF MOLASSES DISTILLERS DRIED SOLUBLES

Constituent	Range of content
	<u>%</u>
Moisture	2-7
Crude protein (N \times 6.25)	7.8-15
Fat	0-0.8
Fiber	0-0.8
Ash	20-35
Vitamins	<u>mg./lb.</u>
Riboflavin	6.8-36
Niacin	22-45
Pantothenic acid	6.8-43
Pyridoxine	13-22
Choline	681-2,724
Biotin	0.9-1.4
Folic acid	1.8-2.3
Vitamin B ₁₂ activity	0.06

TABLE VIII

ESSENTIAL AMINO ACID COMPOSITION OF MOLASSES DISTILLERS DRIED SOLUBLES

Constituent	In sample A ^a	In sample B ^b
	<u>%</u>	<u>%</u>
Crude protein (N \times 6.25)	7.8	11
Arginine	0.04	0.07
Histidine	0.04	0.06
Isoleucine	0.12	0.21
Leucine	0.15	0.31
Lysine	0.07	0.08
Methionine	0.06	0.06
Phenylalanine	0.11	0.15
Threonine	0.14	0.21
Tryptophan	0.03	0.07
Valine	0.18	0.25

^a C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).^b Analyses supplied by Publicker Industries Inc.

of feed being made. Poultry feeds can usually carry quantities up to 2½% of the ration, whereas hog and calf concentrates call for levels up to as much as 5% of the concentrate. The condensed product has been used in dairy feeds and specialty formulas to extend corn or beet molasses and provide animals with some of the nutrients found in cane products.

Both the dried and condensed products have been used to improve the friability and condition of pelleted feeds. There also has been a trend to include both ingredients as a source of unknown growth factors to replace more expensive materials such as animal liver meal and special fermentation products in the manufacture of high-energy rations.

e. Storage and Handling

Molasses distillers dried solubles are usually packed in multiwall paper bags with asphaltic barriers. When properly packed and stored, the product can be kept in dry cool places for as long as six months without caking. Most users order sufficient material to take care of their needs for 60 days.

The condensed material is shipped in 50-gallon steel drums and tank cars. The product is handled in the same types of equipment as is blackstrap molasses, although condensed material has better flow characteristics than does blackstrap molasses.

2. Butyl Fermentation Solubles

a. Raw Materials and Methods of Manufacture

Butanol-acetone fermentation processes have been described in detail elsewhere (44, 45). Although both starch and saccharine materials may be used to produce butyl alcohol and acetone, current prices favor molasses as a raw material.

The problem of disposing of molasses fermentation products from the production of butyl alcohol and acetone is somewhat different from that of ethyl alcohol. In the butyl process, the carbohydrate tolerance of the microorganism is much lower than that of the yeast cell in the ethyl alcohol process. This means that, when the same raw material is used, there is a greater percentage of water in butyl stillage than in the stillage from ethyl alcohol fermentation. Since the types of microorganisms used in the butyl process are sensitive to high concentrations of sugars and salts, there has been a tendency to require either high-test cane molasses or mixtures of high-test and blackstrap molasses as raw materials. Some firms have had success with corn molasses (hydrol) for butyl operations where prices are lower than for cane products. Standard methods are employed for evaporating and drying the end product. From molasses 2 to 3 pounds of dried butanol-acetone stillage are recovered per pound of mixed solvents (or per 0.59 gallon of molasses processed), and from grain 1.1 to 1.3 pounds of dry residue

are recovered per pound of solvents produced (46). Butyl solubles cannot be evaporated to as high a solids content as the ethyl product because of the tendency of the butyl product to solidify.

b. Production (United States)

The quantities of dried butyl molasses fermentation solubles produced in the United States have varied with the competitive positions of butyl alcohol from fermentation and synthetic sources. The trend in recent years has been in favor of the more economic synthetic product.

TABLE IX
TYPICAL PROXIMATE AND VITAMIN ANALYSIS OF DRIED BUTYL MOLASSES
FERMENTATION SOLUBLES

Constituent	Range of content
	<u>%</u>
Moisture	2-8
Crude protein (N \times 6.2)	12-26
Fat	0-0.8
Fiber	0-0.8
Ash	13-35
Vitamins	<u>mg./lb.</u>
Riboflavin	36-227
Niacin	27-68
Pantothenic acid	32-136
Pyridoxine	14-28
Choline	681-2724
Biotin	0.05-0.1
Folic acid	1.8-3.6
Vitamin B ₁₂ activity	0.04-0.08

Before World War II as much as 15,000 tons of dried butyl molasses fermentation solubles were produced annually. At the present time, just a small fraction of this tonnage is made.

c. Composition

Analyses of butyl fermentation solubles are shown in Tables IX and X (47). The variation in range of analyses listed for dried butyl molasses fermentation solubles is mainly accounted for by the kind of raw materials and the amount of re-fermentation. "Backslopping" can be used only to a limited extent in butyl fermentations because of organism sensitivity and contamination possibilities. Generally speaking, more riboflavin and pantothenic acid are synthesized in butyl-acetone than in

TABLE X
ESSENTIAL AMINO ACID COMPOSITION OF BUTYL FERMENTATION SOLUBLES

Constituent	Molasses butyl solubles		Corn butyl solubles ^c
	In sample A ^a	In sample B ^b	
	%	%	%
Crude protein (N \times 6.25)	20	25.1	30
Arginine	0.45	0.47	0.7
Histidine	0.23	0.26	0.5
Isoleucine	0.86	1.30	1.8
Leucine	1.10	1.13	2.2
Lysine	0.68	0.95	1.2
Methionine	0.28	0.41	0.4
Phenylalanine	0.56	0.72	1.0
Threonine	0.64	0.97	0.5
Tryptophan	0.19	0.19	0.2
Valine	0.93	1.21	1.7

^a Analysis supplied by Publicker Industries, Inc.

^b C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).

^c W. Baumgarten, A. N. Mather, and L. Stone, *Cereal Chem.* **23**, 135 (1946).

ethyl alcohol fermentations. The final end product from butyl alcohol fermentations is also higher in protein.

d. Uses in Feeding

Dried solubles from butyl alcohol fermentation of molasses and grain are used in manufactured feeds as a source of riboflavin, pantothenic acid, and unidentified growth factors. A level of 2 to 3% is commonly used in high-energy broiler feeds and up to 5% in hog concentrates.

e. Storage and Handling

Dried butyl molasses fermentation solubles are hygroscopic and must be packed in multiwall paper bags with asphaltic barriers. With proper packing and storage in a dry cool place the dry material can be free-flowing after storage for as long as six months. Care must be exercised in coating the stitches with appropriate sealing tape in the bagging operation so as to prevent moisture from entering the bag through the holes made by the stitching process. Bags should not be stacked too high, since the pressure can aggravate the caking tendency. Dried butyl grain solubles are less hygroscopic.

V. ANTIBIOTICS, VITAMINS, AND GROWTH FACTORS

1. General Information

A number of recently developed fermentation products, including vitamin B₁₂, riboflavin, and antibiotics, have found wide application in commercial feed formulations. Some of these products are made by primary fermentation, i.e., specifically for end use in feedstuffs; others are coproducts or by-products of pharmaceutical manufacture. (See Langlykke *et al.* (48), for the fermentation substrates used.) Antibiotics are added to feedstuffs to maintain animal health, to promote

TABLE XI
UNITED STATES PRODUCTION AND SALES OF VITAMINS AND ANTIBIOTICS^a

Year	Feed riboflavin			Vitamin B ₁₂ , all grades			Antibiotics as feed additives		
			Total			Total			Total
	Pro- duced	Sold	sales value	Pro- duced	Sold	sales value	Pro- duced	Sold	sales value
	1000 lb.		1000 dollars	1000 lb.		1000 dollars	1000 lb.		1000 dollars
1951				84	48	11,000	236	196	17,532
1952				94	61	5,599	258	172	16,962
1953				387	191	14,270	434	391	19,423
1954	181 ^b	118	3,376	422	292	18,894	479	562	25,871
1955	175	123	3,065	488	357	20,614	520	553	26,105

^a Source: Annual Reports on United States Production and Sales of Synthetic Organic Chemicals, U.S. Tariff Commission.
^b Total United States production by fermentation and chemical synthesis during 1954 was 278,000 pounds.

growth, and to improve feed efficiency (49). Vitamin B₁₂ and riboflavin produced by fermentation have greatly increased the availability of these essential vitamins for feed supplementation and, in turn, have permitted much more extensive utilization of vegetable proteins in poultry and swine feeds. Since vitamin and antibiotic concentrates are employed at relatively low levels in rations, they have only a minor effect on the composition of the diet.

2. Antibiotics and Antibiotic Residues

Practically all commercial poultry feeds, about 75% of swine feeds, and certain ruminant feeds contain antibiotics (50). Table XI summarizes the production and sales value in the United States of feed anti-

biotics during the years 1951 through 1955. The most widely employed antibiotics for this purpose are oxytetracycline, chlortetracycline, penicillin, and bacitracin. The analysis of a bacitracin feed additive is shown in Table XII.

Residues resulting from the production of various antibiotics also are used in feedstuffs. Penicillin residues have been found to be rich in

TABLE XII
PROXIMATE AND AMINO ACID ANALYSES OF COMMERCIAL PENICILLIN MYCELIUM,
A BACITRACIN CONCENTRATE, AND A VITAMIN B₁₂ SUPPLEMENT

	Penicillin mycelium ^a	Bacitracin concentrate ^b	Vitamin B ₁₂ supplement ^b
Bacitracin, g./lb.	—	5	—
Vitamin B ₁₂ , mg./lb.	—		6
<i>Proximate analysis</i>	<i>%</i>	<i>%</i>	<i>%</i>
Moisture	1.0	5	5
Crude protein (N × 6.25)	32.3	38	40
Fat	5.5	4	3
Fiber	0.1	8	7
Ash	18.4	15	12
<i>Essential amino acid analysis</i>			
Arginine	1.34	0.74 ^c	0.97 ^c
Histidine	0.67	0.50	0.34
Isoleucine	1.08	1.41	1.28
Leucine	1.84	1.47	1.46
Lysine	0.98	1.33	1.18
Methionine	0.61	0.42	0.41
Phenylalanine	1.14	1.04	0.83
Threonine	1.28	0.96	1.14
Tryptophan	0.71	0.26	0.23
Valine	1.32	1.21	1.36

^a Analysis supplied by Abbott Laboratories, North Chicago, Illinois.
^b Proximate analysis and samples for amino acid determinations supplied by Commercial Solvents Corp., Terre Haute, Indiana.
^c Taken from C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956). These data have been recalculated on the basis of guaranteed potency of vitamin B₁₂ or antibiotic.

certain B vitamins, notably pantothenic acid (51) and to contain an unidentified growth factor(s) for poultry (52–54). The mold mycelium is separated by filtration as the first step in the recovery of penicillin and is dried by some producers as a feed supplement. The composition of one commercial penicillin by-product is shown in Table XII.

Streptomycin fermentation (*Streptomyces griseus*) is characterized by the synthesis of an appreciable amount of vitamin B₁₂ (55). Both

feed supplements and pharmaceutical grades of vitamin B₁₂ may be recovered as by-products of this fermentation. Unidentified chick growth activity also has been observed in streptomycin residues (54).

3. Vitamin B₁₂

Primary fermentation methods utilizing *Streptomyces olivaceous* (56, 57), *Propionibacterium* species (58), and other fungi (59) are the principal sources of vitamin B₁₂. Increasing quantities of the vitamin are being used in the feed trade, as indicated in Table XI. The proximate analysis and amino acid content of a typical vitamin B₁₂ feed supplement are shown in Table XII.

4. Riboflavin

More than half the riboflavin produced in the United States is used in feedstuffs (Table XI). This vitamin is made by chemical synthesis and by aerobic fermentation with either of two yeast-like fungi. The fermentation processes already have been described in detail (60, 61). Distillers grains and solubles, malt sprouts, and acetone-butanol residues are used as carriers of feed riboflavin.

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CHAPTER 28

MILLING FEEDS

R. D. SEELEY

I. INTRODUCTION

The feeds reviewed in this chapter are those obtained as by-products in the wet milling of corn and sorghum grain and the dry milling of rice. The corn feeds are corn gluten feed, corn gluten meal, corn oil meal (corn oil cake meal), steepwater (corn solubles), zein-extracted corn gluten, hydrol, and xanthophyll oil. The sorghum feeds are sorghum gluten feed, sorghum gluten meal, and sorghum oil meal (sorghum oil cake meal). The two feeds obtained from rice are rice bran and rice polishings.

Hominy feed, a by-product feed containing 10 to 11% protein from the dry milling of corn, is not covered in this chapter. Its estimated production is about half that of the combined corn wet milling feeds. In 1950 the average price of hominy feed, which is practically equal to ground corn in feeding value, was \$62.05 per ton, and in 1955 it was \$50.20 per ton (bagged, Chicago) (1, 2).

II. FEEDS FROM THE WET MILLING OF CORN

1. Production and Price

The primary products of the corn refining industry are starch or starch derivatives (dextrin, dextrose, corn syrup) for food and industrial use. From the germ is recovered corn oil which is used primarily for food purposes. Several products of lesser volume, such as zein, inositol, and monosodium glutamate, are produced as additional by-products of the primary manufacture of starch. The major portion of the residuals from the corn refining industry are converted into by-product feeds for the farmer and the feed industry.

The corn refining industry in the United States has more than doubled the volume of corn ground in the past 25 years (Table I) (1, 2). This added volume has been reflected in over a 100% increase

in the production of corn gluten feed and meal. The 1,004,600 tons of corn gluten feed and corn gluten meal produced in the 1954-55 season represent approximately 6% of the major grain by-product feeds prepared, exceeded in tonnage only by the production of cottonseed meal, soybean oil meal, and wheat millfeeds, and equal to alfalfa leaf meal. The production statistics for corn gluten feed and corn gluten meal are reported as combined totals, but the corn gluten meal, which represents the higher protein fraction (41% protein), is approximately 25 to

TABLE I
PRODUCTION AND PRICE STATISTICS ON SOME WET MILLING
BY-PRODUCTS IN THE UNITED STATES^{a,b}

Year	Corn ground (1000 bushels)	Production of corn gluten feed and meal (1000 short tons)	Average wholesale price of corn gluten feed ^c (dollars/ton)
1921	67,898	497.3	31.30
1925	82,780	634.7	34.90
1930	66,489	541.1	24.60
1935	75,826	588.0	23.50
1940	100,750	758.6	23.15
1945	111,732	801.7	48.85
1950	133,191	965.6	52.05
1954-55 ^d	138,568	1004.6	48.40 (1955 av.)

^a U.S. Agr. Marketing Service, Div. of Agr. Econ., *U.S. Dept. Agr. Stat. Bull.* **159** (1955).

^b U.S. Agr. Marketing Service, Feed Situation, FDS-149-155 (1954-55).

^c Bagged, Chicago.

^d October 1954-September 1955 season.

30% of the total. Based on an average production of 1.8 pounds of corn oil meal (corn oil cake meal) per bushel of corn ground, the amount of corn oil meal produced in the 1954-55 season was 124,000 tons. Of this total, however, a large portion of the corn oil meal was not sold separately but was incorporated as part of the corn gluten feed; most solvent-extracted corn oil meal is sold in this manner. The volume of the zein-extracted corn gluten, xanthophyll oil, and hydrol which result from specialized types of production by some corn refining companies is minor compared to that of gluten feeds and corn oil meal.

Wholesale prices of gluten feeds are influenced by the supply and demand of the by-product feeds from other sources which are produced in larger volumes; the average prices per ton in the United States at five-year intervals from 1921 are recorded in Table I (1, 2). The highest average price for any year during the past twenty-five years was \$74.45 in 1947; the price during 1955 was \$48.40. Corn gluten

meal, which contains almost double the amount of protein of corn gluten feed, is correspondingly higher in price; the average price in 1950 and 1954 was \$72.00 and \$78.00 per ton, respectively.* Corn oil meal generally sells at approximately \$3.00 per ton above corn gluten feed prices.

The major portion of steepwater solubles, which are the corn solubles produced by the wet milling of corn, are combined with other residuals to form corn gluten feed. Little, if any, at the present time is sold for feeds as steepwater solids itself. Appreciable amounts are sold separately to the fermentation industry for the production of antibiotics,

TABLE II
ESTIMATED WORLD PRODUCTION OF CORN GLUTEN FEED
AND CORN GLUTEN MEAL FOR 1955^a

Area	Production (metric tons)
South America	43,000
North America (outside the United States)	49,000
Europe	183,000
Other countries	28,000
U.S.S.R.	No data

^a E. E. Daggy, Corn Products Refining Co., New York, private communication.

vitamins, and other fermentation products at a price of approximately \$100.00 per ton (dry solids).

Zein-extracted corn gluten is a by-product of zein manufacture and is not sold as a separate feed ingredient at the present time, although its use is under investigation. No price or production data are available.

Xanthophyll oil, a by-product of zein manufacture, is marketed at approximately 15 cents per pound. Hydrol, so-called feeding corn sugar molasses, sells for approximately \$20.00 per ton (F.O.B. mill) but often is mixed with other feed residuals and sold as "sweetened feeds."

Production data on by-product feeds are not so available elsewhere as in the United States; the data presented in Table II are private estimates of the production of by-product feeds from the wet milling of corn in several countries. The production of gluten feeds in Europe in 1955 was estimated at 183,000 metric tons, which is less than 20% of the production in the United States for the 1954-55 season. The U. S. Department of Agriculture does not list any import figures for corn gluten feeds.

* Bradley & Baker, St. Louis, Missouri, private communication.

2. Method of Manufacture

Yellow corn is used by the corn refining industry primarily because it is in the greatest supply. The manufacturing practices developed by the wet milling industry are based on the structure of the corn kernel,



FIG. 1. Transverse section of a corn kernel. (Courtesy of Corn Industries Research Foundation.)

which, as shown in Fig. 1 (3), consists of a hull and tip cap, a shallow layer of gluten just under the hull, the endosperm, and the germ. In the transverse section of the corn kernel in Fig. 1, the large, well-defined center portion is the germ; the darker area bulging from the side to the center is the horny starch layer of the endosperm; the large white portion at the top or dented end and extending around the germ

is the soft starch portion of the endosperm; and the area below the germ at the bottom of the kernel is the tip cap.

The protein of the corn kernel is distributed as follows: 74.8% in the endosperm, with most of the protein concentrated in the horny

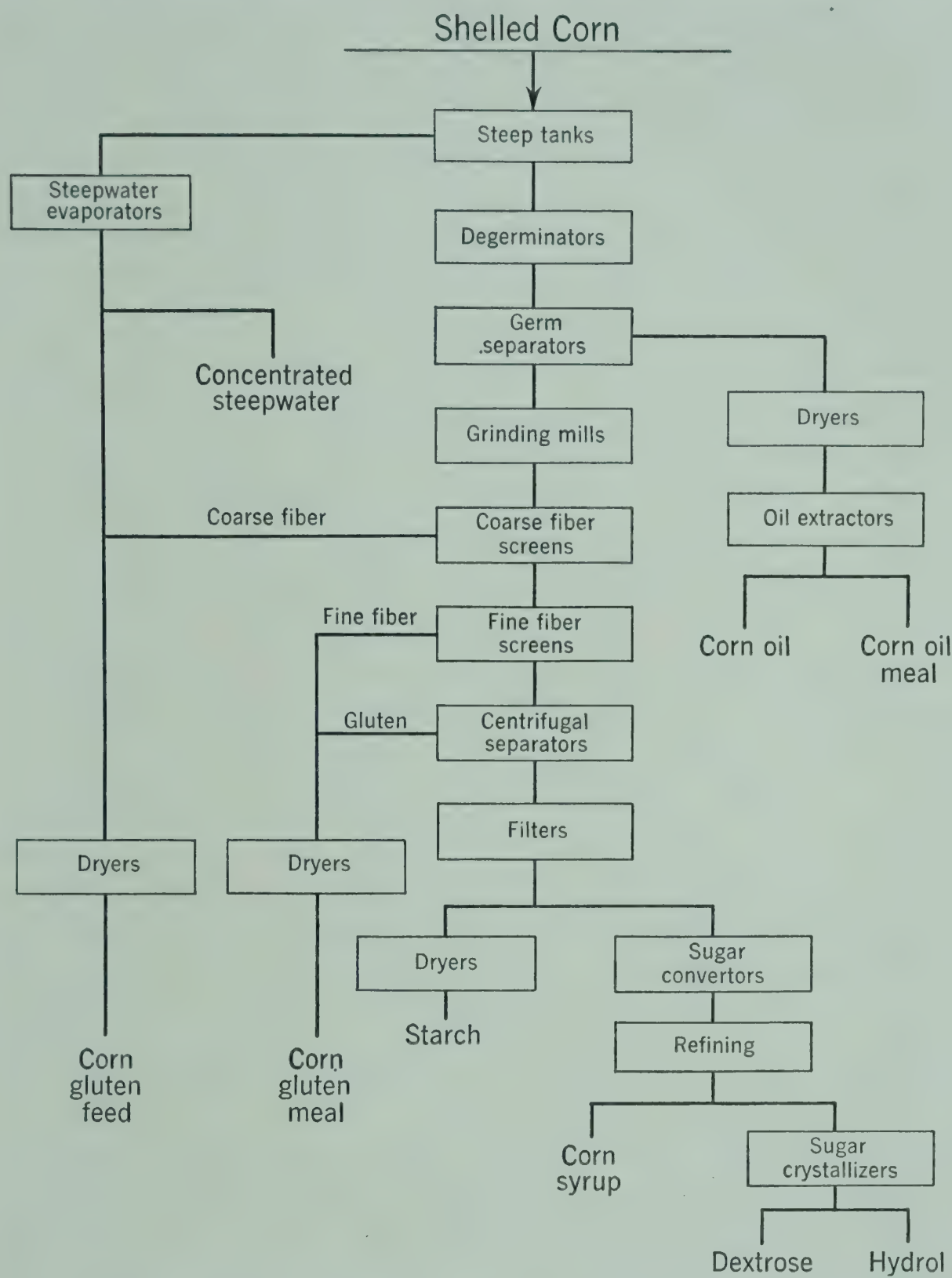


FIG. 2. Diagram of the wet milling process of corn. (Courtesy of Corn Industries Research Foundation.)

starch portion; 22.4% in the germ; and 2.8% in the bran and tip cap (4). Eighty-four per cent of the oil is found in the germ.

The general process used in the wet milling of corn is shown diagrammatically in Fig. 2 (3). Shelled corn after cleaning is steeped in tanks for approximately 40 hours at a temperature of 115 to 130°F. The water used in this steeping operation initially contains approxi-

mately 0.2% sulfurous acid, which helps to reduce and inhibit the growth of microorganisms, softens the hull, and loosens the protein-starch complex for later separation. Nitrogenous material and minerals from the corn kernel are dissolved into the water during the steeping operation. This steepwater containing the corn solubles is then pumped from the tanks to evaporators where it is concentrated to approximately 50% solids, which contain approximately 44 to 48% protein ($N \times 6.25$) on a dry solids basis. The steepwater is usually mixed with other residuals from the process to produce feeds or is taken off in drums or tank cars for special uses. The steeped corn then passes through attrition mills (Foos) where the germ portion is freed from the rest of the kernel. In the germ separators, the germ is removed by flotation and dried, after which the oil is extracted. Corn oil may be removed from the germ by solvent extraction or by screw-pressing, the fraction left after oil removal being the by-product called corn oil meal or corn oil cake meal, a feed containing at least 20% protein.

The mixture of starch, gluten, and hulls is then finely ground in buhr mills and passed through washing screens. Two fractions of fiber are removed at this time: the first is the coarse fiber, which is generally mixed with steepwater solids from the steep tanks and passed through a dryer to become part of corn gluten feed, which is marketed with a minimum of 21% protein; next is the fine fiber, which is removed after passage over the fine fiber screens. The starch and gluten, after the removal of the coarse and fine fibers, pass through centrifugal separators which remove the corn gluten protein, a fraction containing approximately 60 to 70% protein. It is mixed with the fine fiber and dried to produce corn gluten meal, which contains a minimum of 41% protein. Corn gluten feed, corn gluten meal, and corn oil meal are the three primary products for the feed industry produced by the wet milling of corn.

A product of high carbohydrate content is produced after the conversion of the starch to dextrose. After repeated crystallization of the dextrose, the final mother liquor (feeding corn sugar molasses) containing residual corn sugar is recovered. This liquor is called hydrol. Newer processes employing ion-exchange methods substantially eliminate the production of hydrol in the course of dextrose manufacture.

3. Corn Gluten Feed, Corn Gluten Meal, and Corn Oil Meal

a. Chemical Composition

From the method of manufacture of corn gluten feed, corn gluten meal, and corn oil meal, it is evident that corn gluten feed contains a

relatively high fiber content and obtains a large percentage of its nitrogen from corn solubles (corn steepwater); corn gluten meal contains less fiber and more corn gluten; and corn oil meal comes off in a separate stream and contains the germ protein. In some mills, the corn oil meal may be blended into the stream producing corn gluten feed.

In Table III is given the average composition of these three feed products as reported by Morrison (5). The protein levels for corn gluten

TABLE III
CHEMICAL AND MINERAL COMPOSITION OF CORN GLUTEN FEED, CORN GLUTEN MEAL,
AND CORN OIL MEAL (SCREW PRESS)^a

Constituent	Corn gluten feed	Corn gluten meal	Corn oil meal
	<u>%</u>	<u>%</u>	<u>%</u>
Protein (N × 6.25)	24.8	42.9	22.3
Fat	2.6	2.0	7.8
Fiber	7.8	3.9	10.3
Nitrogen-free extract	49.8	40.1	49.0
Ash	6.4	2.5	2.3
Calcium	0.48	0.15	0.06
Phosphorus	0.82	0.36	0.56
Potassium	0.54	—	—
Sodium	0.70	0.08	—
Chlorine	0.33	—	—
Sulfur	0.09	—	—
Magnesium	0.57	0.08	0.20
Iron	0.045	0.045	0.032
	<u>mg./lb.</u>	<u>mg./lb.</u>	<u>mg./lb.</u>
Manganese	11.2	3.9	7.3
Copper	20.8	12.7	5.9

^a F. B. Morrison, "Feeds and Feeding," 21st ed. Morrison, Ithaca, New York, 1954 (c 1948).

feed averaged 24.8%. There are some variations in protein content, depending on the sources of corn gluten feed, and on different manufacturing practices. Most of the corn gluten feed sold today in the United States contains a guarantee of 21% protein, whereas several years ago it was set at 23% and 25%. In a few instances where corn-processing plants do not make corn gluten meal, the protein content of the corn gluten feed may average between 26 and 30% because it contains the corn gluten fractions which would ordinarily go into gluten meal. The fat content of screw-pressed corn oil meal is relatively high (7.8%), whereas solvent-extracted corn oil meal contains only 1.5% fat. The fiber content, a property which affects the practical use of these feed products, averages 7.8% in corn gluten feed; this feed on

the market usually is guaranteed to contain less than 10% fiber. The fiber content of corn gluten meal averages 3.9%, which makes this an acceptable product for use in low-fiber feeds such as for the poultry industry. Corn oil meal has the highest fiber content, averaging 10.4%.

b. Minerals

The mineral analyses shown in Table III were the averages reported by Morrison (5). There is no great difference in mineral content among the three feed products except that the corn gluten meal

TABLE IV
VITAMIN COMPOSITION OF CORN GLUTEN FEED, CORN GLUTEN MEAL,
AND CORN OIL MEAL^{a,b}

Vitamin	Corn gluten feed (mg./lb.)	Corn gluten meal (mg./lb.)	Corn oil meal (mg./lb.)
Thiamine	0.5	0.2	0.1
Riboflavin	1.1	0.8	1.2
Niacin	31.1	24.8	17.1
Pantothenic acid	6.1	3.0	1.3
Pyridoxine	6.7	3.6	2.7
Choline	1270	264	490
Biotin	0.2	0.1	0.1
Folic acid	0.2	0.1	0.1
Inositol	2800	900	1532
Vitamin K, units	227	125	264
Vitamin A, USP units	2725	9589	310

^a E. E. Daggy, Corn Products Refining Co., New York, private communication.

^b A. W. Turner, A. E. Staley Manufacturing Co., Decatur, Illinois, private communication.

contains less of the various minerals because of its lower total ash content. There are some variations in calcium content in corn gluten feed due to differences in manufacturing processes. In some corn processing plants, calcium salts are used in the production of by-products such as inositol and monosodium glutamate, and this use results in the higher calcium content of corn gluten feed; calcium levels for corn gluten feed vary from 0.15 to 1.0%. Phosphorus, an important mineral in animal nutrition, is considerably higher in corn gluten feed than in corn gluten meal.

c. Vitamins

Vitamin analyses for yellow corn reported in Table IV were the average values taken from the laboratories of two corn processing plants. Only insignificant differences were reported in the content of

B-complex vitamins of these three feeds. Relatively small amounts of B-complex vitamins are supplied to animals from these feed sources, with the inositol and choline levels being highest. There were large differences in the vitamin A activity among the feeds. Vitamin A does not occur as such in yellow corn, the vitamin A activity resulting from the conversion in the animal of the provitamins cryptoxanthin and β -carotene. The greatest amount of this vitamin activity is found in corn gluten meal, with an average content of approximately 9500 USP units per pound, varying from 4000 USP units per pound for one sample to 15,000 USP units per pound from another commercial source. Morrison (5) reports an average value of over 16,000 USP units per pound. The differences reported from various sources will be the result of commercial practice, as the vitamin A activity is closely associated with the amount of corn gluten fraction which makes up the feed. The levels of vitamin A activity in corn gluten feed were approximately 2700 USP units per pound. Morrison (5) reported an average value of 10,000 USP units per pound. The vitamin A activity of the corn gluten feed fraction would be much greater from corn processing plants where corn gluten meal is not marketed than in plants which remove corn gluten meal as a separate product. Corn oil meal contains a significantly lesser amount of vitamin A activity, the average value being 310 USP units per pound. There is no vitamin A activity in corn solubles (steep-water) (6).

Beta-carotene and cryptoxanthin are two of three carotenoids found in corn, the other being zeaxanthin (7). The carotenoids are also valuable in feeds because they impart a yellow color to the shanks, beaks, skin, and fat of poultry, which improves the appearance of dressed poultry (8, 9). Xanthophyll oil, which will be discussed later, is a concentrated source of these pigments.

d. Amino Acid Composition of Corn Gluten Feed, Corn Gluten Meal, and Corn Oil Meal

The literature contains many amino acid determinations on corn and corn by-product feeds (10-14). The analyses by Lyman (15), recorded in Table V, were selected because they are recent ones and were determined at the same time in one laboratory on these three closely related feed products. The actual amino acid content of corn gluten feed and corn oil meal is much less than corn gluten meal because the protein content of the latter is approximately twice that of the others, but in evaluating a protein for quality, the amino acid analyses calculated for the crude protein as reported in Table V are a better basis of comparison.

TABLE V
AMINO ACID ANALYSES OF CORN GLUTEN FEED, CORN GLUTEN MEAL,
CORN OIL MEAL, AND SOYBEAN OIL MEAL

Amino acid	Corn gluten feed ^a		Corn gluten meal ^a		Corn oil meal ^a		Soybean oil meal ^b	
	In the sample (%)	In	In the sample (%)	In	In the sample (%)	In	In the sample (%)	In
		crude protein ^c (%)		crude protein ^c (%)		crude protein ^c (%)		crude protein ^c (%)
Arginine	1.0	4.3	1.5	3.3	1.4	6.3	2.3	4.7
Histidine	0.7	3.0	1.0	2.2	0.7	3.0	1.1	2.2
Isoleucine	0.8	3.5	2.0	4.4	0.9	3.9	2.2	4.6
Leucine	2.3	10.2	7.5	16.5	1.8	8.2	2.7	5.5
Lysine	0.7	3.2	1.0	2.1	1.0	4.7	3.0	6.2
Methionine	0.5	2.1	1.2	2.7	0.4	2.0	0.3	0.6
Phenylalanine	0.9	3.8	2.8	6.1	0.9	4.1	2.2	4.6
Threonine	0.8	3.5	1.6	3.6	0.9	3.9	1.8	3.6
Tryptophan	0.2	0.7	0.2	0.5	0.2	1.0	0.6	1.2
Valine	1.3	5.5	2.4	5.2	1.3	5.9	2.4	4.9
Crude protein (N × 6.25)		22.9		45.7		22.2		48.7

^a C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).

^b H. H. Williams, *Proc. Cornell Nutrition Conf. for Feed Mfrs.* p. 76 (1950).

^c Calculated as grams of amino acid per 16 g. of nitrogen.

McCollum *et al.* (16) reported that zein, a protein from corn, was low in content of cystine and contained no lysine or tryptophan. From animal studies, Mitchell and Smuts (17) and Hogan (18) determined that whole corn was also deficient in lysine and tryptophan. The apparent low biological value of corn gluten meal indicated that this same deficiency occurs in this feed product (19, 20), and these observations are confirmed by the amino acid analyses reported in Table V where, in the crude protein, the tryptophan content of corn gluten feed and corn gluten meal is only 0.70% and 0.52%, respectively, and the lysine content 3.24% and 2.10%, respectively. The lysine content of the gluten feeds is less than one-half that of soybean oil meal; the tryptophan level is 60% or less of that in soybean oil meal; leucine is 100 to 200% higher for corn gluten feed and meal; and the methionine content is over 200% higher. These data demonstrate that the primary deficiencies of gluten feeds can be balanced by other feed-protein sources such as soybean oil meal, which is one of the most available of the feed concentrates. Through amino acid supplementation, the overall protein quality of mixtures can be increased by balancing those

limiting amino acids of corn gluten feeds. Compared to soybean oil meal, the gluten feeds have equivalent levels of the other eight essential amino acids.

Corn oil meal, which contains the germ protein of corn, has been reported by Mitchell and Beadles (21) to have a biological value in rats of 77.6% (nitrogen balance method), which is equal to beef round, a high-quality animal protein, and superior to soybean oil meal. Similar high nutritive values for corn germ protein have been observed by Jones and Widness (22) and by Schulz and Thomas (23). The higher quality of the protein of corn oil meal over corn gluten feed and corn gluten meal is reflected in the lysine and tryptophan contents, which are significantly higher.

e. Uses in Feeds

(1) *Corn gluten feed.* The fiber content of corn gluten feed, which is generally higher than 7%, has restricted its use in low-fiber poultry feeds. This feed product is widely used, however, to provide a portion of the protein in non-ruminant rations. Ewing (24) reports that, although the general practice by poultrymen and feed manufacturers is to keep rations below 7% in fiber, levels of 8 to 9% can be used without affecting chick growth, mortality, or feed consumption.

Slinger *et al.* (25) reported that corn gluten feed in growing chick rations can be added in an optimal amount of 10% with 6% meat meal. In laying and breeding rations, up to 16% was used in the ration, replacing one-half of the meat meal. The use of corn gluten feed in poultry rations provides a cheap source of protein with an additional supply of provitamin A and xanthophyll pigments, but, as can be seen from the amino acid analyses, it should not be the chief protein supplement for poultry. Similarly, corn gluten feed, when mixed with tankage, has been used for fattening fall pigs, but as a sole supplement corn gluten feed produced poor results (26). This feed is not very palatable to swine and should be used at low levels.

Corn gluten feed is most widely used for feeding dairy cows. Morrison (27) reports good results when corn gluten feed was fed with corn silage and mixed hay low in content of legumes. In a comparison of various protein supplements for the dairy cow, corn gluten feed was equal to linseed oil meal, peanut meal, and soybean oil meal (28). For fattening lambs, corn gluten feed was not as good as corn (29, 30).

(2) *Corn gluten meal.* Corn gluten meal, which contains a maximum of 4.0% fiber, has a wider usage in poultry rations because of its ready acceptance for incorporation in low-fiber feeds. Corn gluten meal is not used as the sole protein concentrate in poultry feeds because of

its deficiency in lysine and tryptophan; however, as shown in Table V, the limiting amino acids, lysine and tryptophan, of corn gluten meal and the higher levels of leucine and methionine balance the amino acid pattern of soybean oil meal so that the combination should result in a superior protein mixture.

Fritz (31) reported that in broiler rations the biological value of corn gluten meal was 21% and of soybean oil meal was 68%. When a 1:1 combination of corn gluten meal and soybean oil meal was fed, the biological value of the mixture of proteins was raised to 86%. The increase was attributed to the mutual supplementation of lysine and methionine between the protein sources. Van Landingham *et al.* (32) reported similar results by nitrogen balance studies in growing chicks; the biological value of corn gluten meal was 28.7% and of soybean oil meal was 76.0%, but when a combination (equal protein basis) of these two protein sources was fed the average biological value was 68.3%. Similar supplementation effects were observed with combinations of corn gluten meal and fish meal or meat scraps, although the biological values were lower as compared to the soybean oil meal-corn gluten meal mixture. Supplementation studies involving corn gluten meal and soybean oil meal also have been reported by others (33, 34). Corn gluten meal can also be used in poultry rations in combination with good-quality animal protein rich in lysine, such as the protein of dried milk and meat scraps (35).

Camp and Couch (36) noted that, when corn gluten meal was substituted at the 5% level for soybean oil meal, not only did the growth rate and feed conversion improve but also the degree of pigmentation increased because of the xanthophyll pigments. Similar improvement in dressed turkeys has resulted from feeding corn gluten meal as 33 to 66% of the protein concentrate of the ration (37). Growth responses in turkeys by substituting corn gluten meal for part of the fish meal, meat meal, or dried milk were improved as in broiler rations (38).

Corn gluten meal as the primary protein source in the feeding of swine produced poor weight gains and protein efficiencies (39). These results could be expected from the amino acid pattern of corn gluten meal, but, as in poultry feeding, corn gluten meal should produce good results if fed in combination with good-quality protein sources such as animal or soybean protein.

Corn gluten meal has been reported to be equal to cottonseed meal and linseed meal in rations for fattening cattle, but it was the least palatable of these protein supplements (40). Palatability was improved when mixtures of these feeds were fed to the cattle. For dairy cattle, corn gluten meal is a satisfactory protein supplement, supplying a so-called "grain factor" or "milk-stimulating" factor (41).

Corn gluten meal has been reported by several investigators (42-46) to be approximately equal to linseed meal, cottonseed, or soybean oil meal for growing lambs.

(3) *Corn oil meal*. This feed by-product from corn, which represents only about 10% of the total feeds produced by the wet milling industry, contains protein with a better balance of amino acids, as shown by amino acid analyses (15) and animal studies (21–23). For poultry rations, corn oil meal is limited in use by its relatively high fiber content. Corn oil meal when used as a major fraction of the protein concentrates in poultry feeds produces poor chick growth, owing to poorer palatability as well as possible deficiencies in protein quality (47). The protein quality of poultry rations containing corn oil meal was improved by including fish meal or skim milk. Although the protein quality of corn oil meal is better than corn gluten meal, good-quality protein must be furnished by other protein concentrates in poultry feeds containing corn oil meal. One factor which may affect feed consumption when corn oil meal is used in rations is its increase in volume after wetting (48). For example, corn oil meal after wetting increased 210% in volume, as compared to 48 to 61% for fish meals, other cereal by-products, and whole grains. This water-absorbing ability of corn oil meal makes it an excellent carrier for such materials as blackstrap molasses for cattle feeds.

Corn oil meal is used in rations for growing and fattening pigs. Geurin *et al.* (39) observed that corn oil meal was equivalent to soybean oil meal for growth. Per pound of protein consumed, corn oil meal was equal to skim-milk protein and better than soybean oil meal for growth, but, as palatability was poor, the actual growth rates on corn oil meal were lowest of the three protein sources. This demonstrates that corn oil meal is a good protein source but must be used at lower levels with other protein feeds so that palatability does not become a limiting factor. Scouring may also result from feeding too high a level (49). It was reported in these experiments that best results were obtained when corn oil meal was fed at no more than one-fourth of the ration.

f. Storage and Handling

As corn gluten feed, corn gluten meal, and corn oil cake meal are generally not held in storage for long periods after milling, there are little data on long-term storage of these by-product feeds. It is reported by Schulz and Thomas (23) that corn oil meal which had been held in storage and assayed in feeding tests with rats had a biological value after three months of 83%; after six months, 79%; and after six years, 66%, indicating a definite decrease in biological value on long-term storage. Corn oil meal may become rancid after prolonged storage because of its relatively high fat content. In poultry rations, fresh corn oil meal was found to be better than the stored product (50).

Mitchell and Beadles (51) tested in rats, by the nitrogen balance method, the biological value of corn stored as whole kernels and as ground meal for periods of 730 and 1020 days at 78°F. with a moisture content of 6 to 12%. There was no deterioration in the biological value after the two- to three-year period, nor was there any change in the true digestibility of the protein sources. This would indicate that a long period of storage of corn in warehouses does not affect the quality of the protein contained in the corn; therefore, it is probably unlikely that the milling by-products from such stored corn would be changed in protein quality.

4. Steepwater

a. Approximate Chemical and Mineral Composition of Steepwater Solids

The analysis of concentrated steepwater solids, as recorded in Table VI, shows this product to contain approximately 52% solids and 48%

TABLE VI
CHEMICAL COMPOSITION OF CONCENTRATED STEEPWATER^{a, b}

Constituent	Content (%)	Constituent	Content (%)
Total solids	52	Potassium	4.4
Nitrogen	7.8	Magnesium	1.9
Lactic acid	26	Sodium	0.2
Carbohydrates as dextrose	2.5	Phosphorus	3.3
Ash	18	Sulfur	0.7
Phytic acid	7.5	Calcium	0.1
Ether extractables	2.0		

^a Dry weight basis.

^b S. A. Watson, Corn Products Refining Co., New York, private communication.

protein ($N \times 6.25$) on a dry solids basis. It also contains approximately 26% lactic acid and 7.5% phytic acid. During the steeping process, there is a certain amount of growth primarily of lactic acid-producing microorganisms in the steepwater. These organisms are destroyed later during concentration of the steepwater. The amount of lactic acid produced varies according to the length of time this "souring" takes place. "Souring" may be allowed to proceed after removal of the steepwater from the corn to produce "soured" steepwater.

Mineral contents of concentrated steepwater solids are also shown in Table VI. These data indicate a low content of calcium.

b. Vitamins and Amino Acids

The vitamin content of steepwater, as recorded in Table VII, is higher than that of the gluten feeds, but in approximately the same proportions. Concentrated corn steepwater provides a source of amino acids and peptides, but the balance of essential amino acids is poor

TABLE VII
VITAMIN CONTENT OF SPRAY-DRIED STEEPWATER^a

Vitamin	Content (mg./lb.)
Thiamine	2.3
Riboflavin	4.9
Niacin	67.5
Pyridoxine	7.3
Pantothenic acid	12.3
Biotin	0.2
Folic acid	1.1
Inositol	6210
Choline	1230

^a B. L. Scallet, Central Research Laboratory, Anheuser-Busch, Inc., St. Louis, Missouri, private communication, 1949.

TABLE VIII
AMINO ACID COMPOSITION OF CONCENTRATED STEEPWATER^a

Amino acid	Content (% of total nitrogen)	Amino acid	Content (% of total nitrogen)
Leucine	5.9	Proline	4.8
Isoleucine	3.4	Phenylalanine	2.0
Valine	3.9	Methionine	1.1
Glutamic acid	8.0	Aspartic acid	1.7
Threonine	3.4	Cystine	1.2
Lysine	4.0	Alanine	27.7
Arginine	8.2	Tyrosine	0.7
Histidine	6.8	Ammonia	12.7

^a E. V. Cardinal and L. R. Hedrick, *J. Biol. Chem.* **172**, 609 (1948).

(Table VIII) (52). There are no values given for tryptophan content, but it is considered to be very low. One characteristic of concentrated steepwater is that the nitrogenous constituents are largely peptides and free amino acids. These amino acids, peptides, minerals, vitamins, and unknown factors, which are poorly utilized when fed as a sole nutrient source to animals, provide a rich medium for the growth of microorganisms. Hence steepwater is widely used throughout the fermentation industry as a nutrient medium for the production of antibiotics

and vitamins; its use in microbiology has been reviewed by Liggett and Koffler (53).

c. Uses in Feeds

Most of the steepwater produced by wet milling of corn is dried with other residuals to make corn gluten feed. It is known that corn steep liquor is excellent for growing propionic acid-producing bacteria. Possibly, the beneficial results of feeding corn gluten feed to ruminants are partly due to this readily available source of nutrients for the

TABLE IX
CHEMICAL ANALYSIS AND AMINO ACID COMPOSITION OF ZEIN-EXTRACTED GLUTEN^a

Component	Content (%)	Amino acid	In crude protein ^b (%)
Moisture	7.2	Arginine	5.0
Protein (N × 6.25)	51.8	Cystine	5.8
Ash	2.8	Glycine	3.5
Fat	0.15	Histidine	3.0
Fiber	3.2	Isoleucine	13.7
Nitrogen-free extract	34.9	Lysine	4.0
		Methionine	4.2
		Phenylalanine	6.0
		Tryptophan	0.55
		Threonine	4.0
		Valine	5.7

^a E. E. Daggy, Corn Products Refining Co., New York, private communication.

^b Calculated as grams of amino acid per 16 g. of nitrogen.

propionic acid-producing bacteria which appear as a large part of the rumen microflora. Although it is not the practice to add concentrated corn steepwater by itself to rations, it might have benefits as a stimulant for ruminant digestion.

The amino acid deficiencies and high ash content, as shown by analysis and results of animal studies (23, 39), discourage the use of corn steepwater as a major source of protein for animal feeds.

5. Zein-Extracted Corn Gluten

a. Proximate Chemical Composition

Zein-extracted corn gluten, which is recovered from corn gluten after the extraction of zein, is a minor by-product of the wet milling industry. Only a limited amount of analytical data has been reported because this product has not yet been made available to the feed industry. Its protein content (Table IX) is higher than that of corn gluten

meal; the fiber content is approximately the same; the fat level is very low.

b. Amino Acids

Zein-extracted corn gluten should be of higher protein quality than corn gluten meal, as the former contains almost one-third more methionine and twice as much lysine (Table IX), and also contains 5.8% cystine. In content of the other essential amino acids, it is either equal to or higher than corn gluten meal, except for tryptophan.

c. Uses in Feeds

This product is not yet available to the feed industry, but its use is under study. Although the supply would be limited, its particular amino acid pattern is of interest. Zein-extracted corn gluten has been substituted for soybean meal in broiler rations on an equiprotein basis up to the 10% level and found to be equal or slightly superior to a soybean oil meal basal ration (36) in promoting growth. In a swine feeding experiment, substitution of 50% of the soybean oil meal with zein-extracted gluten produced weight gains equivalent to those obtained on a ration with soybean oil meal as 100% of the protein concentrate.

6. Hydrol (Feeding Corn Sugar Molasses)

Hydrol, which is the concentrated mother liquor after the crystallization of dextrose, contains approximately 26% moisture, 9% ash, 0.2% protein, and 65% total sugars. The carbohydrate fraction consists of about 63% dextrose, 29.4% disaccharides, and 7.5% higher oligosaccharides (54). The disaccharide fraction contains 15.2% isomaltose, 5.0% gentiobiose, 2.8% maltose, 1.7% α,α -trehalose, and 4.7% other sugars.

Hydrol is equal to cane molasses in its content of total digestible nutrients. In a feeding experiment with calves, Ward *et al.* (55) found that corn sugar replaced 10 to 20% of a standard grain mixture and produced equal growth responses. Camp and Stephenson (56) reported that in a basal diet of soybean oil meal and ground yellow corn supplemented with the required minerals and vitamins and aureomycin, hydrol increased chick growth and raised the feed utilization by 0.2 pound per pound of feed. The energy levels were essentially the same. These authors tentatively concluded that hydrol might contain an unidentified growth factor.

Annual production of hydrol in the United States is estimated at approximately 100,000 tons.

7. Xanthophyll Oil

Xanthophyll oil is a reddish-brown oil which is recovered from corn gluten in the process of extraction of zein. The supplies of this by-product are small, owing to the limited production of zein. The oil contains fatty acids, glycerol, unsaponifiable sterols, and approximately 0.4% xanthophyll pigments (8), of which the principal one is zeaxanthin (57). The vitamin A activity of this oil is a minimum of 400 International Units per gram. The oil is useful as a poultry feed supplement to increase normal pigmentation, imparting a desirable yellow color in the skin, which enhances the marketability of the poultry (9). Recommended proportions are 0.25 to 3.0% in poultry feeds; best results are obtained at 2% (58).

III. FEEDS FROM THE WET MILLING OF GRAIN SORGHUM

1. Methods of Manufacture

The manufacturing process for producing grain sorghum (also known as milo) feeds is essentially the same as used for the wet milling of corn, and the same types of feed are obtained—namely, sorghum gluten feed, sorghum gluten meal, and sorghum oil meal (sorghum oil cake meal).

2. Production and Price

Grain sorghum feeds produced by the wet milling process have been available only in recent years. One plant in Texas produces starch from grain sorghum by the wet milling process. This plant, which started operations in 1949, produced approximately 33,000 tons of sorghum gluten feed and meal in 1950 and 60,000 tons of these feeds in 1955.* The average price in 1955 for sorghum gluten feed and sorghum gluten meal was \$37.79 and \$52.28 per ton, respectively. In this plant the sorghum oil meal is added to the sorghum gluten feed and is not sold as a separate product.

3. Sorghum Gluten Feed, Sorghum Gluten Meal, and Sorghum Oil Meal

a. Chemical Composition

The chemical composition of sorghum gluten feed and meal is similar to that of corn gluten feed and meal (Table X) (59), except that the ash content of sorghum gluten meal is only 0.7%. The sorghum gluten feed and meal contain 25.0% and 41.7% protein, respectively.

* E. E. Daggy, Corn Products Refining Co., private communication.

b. Minerals and Vitamins

The similarity between the corn gluten feed products and the sorghum gluten feed products in vitamin and mineral content is also evident from the limited analyses available (Table X). The most striking difference is the absence of vitamin A activity and the xanthophyll

TABLE X
CHEMICAL, VITAMIN, AND MINERAL COMPOSITION OF SORGHUM GLUTEN FEED
AND SORGHUM GLUTEN MEAL^a

Component	Sorghum gluten feed	Sorghum gluten meal
	<u>%</u>	<u>%</u>
Crude protein (N × 6.25)	25.0	41.7
Fat	3.4	4.1
Fiber	6.3	2.8
Nitrogen-free extract	48.4	40.3
Ash	7.7	0.7
Calcium	0.09	0.02
Phosphorus	0.59	0.17
	<u>mg./lb.</u>	<u>mg./lb.</u>
Thiamine	2.6	—
Riboflavin	5.4	1.9
Panthothenic acid	9.8	2.4
Niacin	45.9	22.4

^a *Tex. Agr. Expt. Sta. Bull.* **743** (1951).

pigments which affect the color of dressed poultry. Sorghum feeds provide little calcium to the animals, but sorghum gluten feed can provide appreciable phosphorus if liberally fed.

c. Amino Acids

The pattern of amino acids in the protein of sorghum gluten feeds, as reported by Lyman (15), resembles closely the amino acid composition of corn gluten feeds (Table XI). Similarly, sorghum oil meal has an amino acid composition approximately the same as corn oil meal. Although sorghum oil meal is not marketed as such, the data are included for comparison. The most significant difference reported by Lyman is the tryptophan content which is slightly higher for sorghum gluten feed and twice as great for sorghum gluten meal when compared to the corresponding corn gluten feeds. The lysine and methionine levels, however, are lower in sorghum gluten meal than in corn gluten meal.

TABLE XI
AMINO ACID COMPOSITION OF SORGHUM GLUTEN FEED, SORGHUM GLUTEN MEAL,
AND SORGHUM OIL MEAL^a

Amino acid	Sorghum gluten feed		Sorghum gluten meal		Sorghum oil meal	
	In the sample (%)	In crude protein ^b (%)	In the sample (%)	In crude protein (%)	In the sample (%)	In crude protein (%)
Arginine	1.0	4.3	1.2	2.7	1.5	6.8
Histidine	0.6	2.7	0.8	1.8	0.7	3.4
Isoleucine	1.0	4.4	2.3	5.1	1.0	4.5
Leucine	2.5	11.4	7.3	16.4	1.9	8.7
Lysine	0.7	3.0	0.6	1.3	0.9	4.0
Methionine	0.4	1.7	0.7	1.6	0.4	1.6
Phenylalanine	1.0	4.6	2.6	5.8	1.0	4.6
Threonine	0.8	3.6	1.3	3.0	0.7	3.4
Tryptophan	0.2	0.8	0.4	1.0	0.2	1.0
Valine	1.3	5.8	2.5	5.7	1.4	6.7
Crude protein (N × 6.25)	22.3		44.5		21.6	

^a C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).

^b Calculated as grams of amino acid per 16 g. of nitrogen.

d. Uses in Feeds

As sorghum gluten feed and meal have been used for animal feeds for only the past six years, the literature on their use is rather limited. In 1950, 63% of the grain sorghum crop in the United States was grown in Texas, where the grain sorghum, unlike corn, is better adapted to the hot, semiarid climate. Grain sorghum provides the bulk of the grain for Texas livestock and poultry feeders (59). A comprehensive review of feeding experiments with these feed products was reported by the Texas Agricultural Experiment Station in 1951 (59). These results will be briefly summarized in the following sections.

(1) *Sorghum gluten feed*. Sorghum gluten feed, because of its high fiber content, has not been used experimentally in broiler and starter rations for poultry. From its amino acid composition it should be comparable to corn gluten feed, but it contains no vitamin A activity.

In rations for fattening steers, sorghum gluten feed is a good feed but is less palatable than cottonseed meal. Best results are produced when it is fed in combination with other, more palatable feeds. The lower palatability of sorghum feeds has been attributed to the presence of tannins, but this is a variable factor, depending on the grain source.

The use of molasses in such feeds would eliminate this problem in sorghum gluten feed which might otherwise have some bitter taste. Sorghum gluten feed is usually fed to dairy cattle at 35% or less of the concentrate ration.

Sorghum gluten feed as the only protein supplement given to sheep already receiving sorghum grain produced unsatisfactory results. Good results were obtained when the sorghum gluten feed constituted two-thirds of a protein supplement which included cottonseed meal and low-grade roughages. As palatability may be a limiting factor with this feed, it is recommended that no more than 30% of sorghum gluten feed be used in the concentrate ration.

Sorghum gluten feed should be used at a low level in protein supplements for swine. At the 30% level, poor results were obtained, attributed to amino acid deficiencies.

(2) *Sorghum gluten meal*. This sorghum feed does not compare favorably with corn gluten meal in poultry rations. The lower levels of arginine, methionine, and lysine in sorghum gluten meal as compared to corn gluten meal must be critical. When fed at a 5% level with 25% soybean meal, it produced no supplementary effect as found with mixtures of soybean oil meal and corn gluten meal. When 10% sorghum gluten meal was added to 20% soybean oil meal, the weight gain was only 71% of that achieved by a 30% soybean oil meal ration. Supplementation of sorghum gluten meal with individual amino acids indicates that unidentified deficiencies other than lysine, arginine, and methionine may exist. Another difference between sorghum gluten meal and corn gluten meal is the relative absence of xanthophyll pigments and vitamin A activity.

Sorghum gluten meal is equal to cottonseed meal as a protein source for cattle, as measured by daily gain, dressing percentage, and carcass grade. Sorghum gluten meal was compared to corn gluten meal in dairy cattle rations and found to have 90% of the value of corn gluten meal. The sorghum gluten meal made up 37% of the concentrate ration, at which level it was equally as palatable as corn gluten meal.

Sorghum gluten meal was compared to cottonseed meal as a protein supplement for sheep and found to be poorer when fed with grain sorghum, cottonseed hulls, and Johnson grass hay, but satisfactory results were obtained when alfalfa hay was fed with the sorghum gluten meal. These data indicate that, with low-grade roughages, a 50-50 mixture of sorghum gluten meal and cottonseed meal in the protein supplement would be satisfactory. The failure of sorghum gluten meal with low-grade roughages is attributed to a lower availability of protein or a shortage of methionine.

The deficiency of essential amino acids in sorghum gluten meal limits the use of this feed in swine rations. It is recommended that the levels be kept below 30% of the protein supplement and not more than 4% of the total ration.

IV. FEEDS FROM THE MILLING OF RICE

1. Production and Price (United States)

The volume of rice millfeeds produced in the United States in the last twenty years has increased markedly: in 1935, production was 88,500 tons; in the 1954-55 season, it had increased to 303,000 tons (Table XII) (1, 2). These by-product feeds are produced in relatively

TABLE XII
PRODUCTION OF RICE MILLFEEDS^{a, b}

Year	Amount (1000 short tons)
1935	88.5
1940	124.8
1945	154.8
1950	187.0
1954-55 ^c	303.0

^a U.S. Agr. Marketing Service, Div. of Agr. Econ., *Stat. Bull.* **159** (1955).
^b U.S. Agr. Marketing Service, Feed Situation, FDS-149-155 (1954-55).
^c October 1954-September 1955 season.

small tonnages but have shown one of the greatest increases over the past twenty years on a percentage basis. The larger percentage of rice millfeeds is in the form of rice bran; the average price in 1955 was \$32.50 per ton, sacked, F.O.B. production point.* The prices for rice polish average approximately \$5.00 to \$7.00 per ton more than rice bran.

2. Method of Manufacture

Rice grain received at the mill is called “rough” rice and is composed of a hull, a seed coat consisting of several layers, a starchy endosperm, and the germ. In the mill, four separate fractions are produced: the milled rice, hulls, bran, and polish. The milled rice is used primarily for human food, the hulls for the manufacture of furfural or for roughage in feeds, and the rice bran and rice polish for animal feeds.

In the milling of rice, the rough rice is cleaned and passed through shellers which remove the hulls. After shelling, the brown rice passes

* Bradley & Baker, St. Louis, Missouri, private communication.

through a scouring step where, mechanically, the bran and germ are progressively removed from the brown rice to form rice bran feed. The rice at this stage retains only a small fraction of the bran, mainly the inner layers. It then passes to a polishing machine, called a brush, which removes the remaining bran. This last bran fraction is sold as

TABLE XIII
CHEMICAL, VITAMIN, AND MINERAL COMPOSITION OF RICE BRAN AND POLISH

Constituent	Bran			Polish
	%			%
Nitrogen	1.53	1.85	1.70	1.8
Crude protein (N × 6.25)	9.6	11.6	10.6	11.0
Lipids	13.7	15.1	10.6	16.4
Ash	12.0	14.3	20.6	13.2
Calcium	1.3	1.7	3.8	0.91
Phosphorus	1.5	1.9	1.1	2.4
Iron	0.02	—	—	0.03
Crude fiber	—	9.6	10.1	—
Moisture	9.8	10.3	11.0	9.8
	mg./lb.			mg./lb.
Thiamine	10.9			10.0
Riboflavin	0.9			1.0
Niacin	153			150
Pantothenic acid	12.6			15.3
Biotin	0.3			0.3
Folic acid	0.7			0.9
Pyridoxine	11.3			9.1
Inositol	2100			2060
Choline	772			463
p-Aminobenzoic acid	0.3			0.3
References	a	b	b	a

^a From M. C. Kik, *J. Agr. Food Chem.* **4**, 170 (1956); M. C. Kik, *Arkansas Agr. Expt. Sta. Bull.* **589** (1957).

^b From K. S. Murti and F. G. Dollear, *J. Am. Oil Chemists' Soc.* **25**, 211 (1948).

rice polish. As can be seen from this process, the rice polish feed is produced in much smaller quantities than rice bran. For a more detailed description, see Kik and Williams (60).

3. Rice Bran and Rice Polish

a. Chemical, Vitamin, and Mineral Contents

The protein, fat, and ash contents of rice bran and rice polish (Table XIII) are approximately equal (61). The fat content is somewhat higher for rice polish but of the same order of magnitude. These

feeds are rich sources of thiamine and niacin. There is a big difference in fiber content—12% for rice bran compared to about 3% for rice polish (5).

b. Amino Acids

The amino acid pattern of these two feeds is quite similar (Table XIV) (61). Rice polish contains a somewhat larger amount of methio-

TABLE XIV
AMINO ACID ANALYSES OF RICE BRAN AND RICE POLISH^a

Amino acid	Rice bran		Rice polish	
	In the sample ^b	In crude protein ^c	In the sample ^b	In crude protein ^c
	%	%	%	%
Arginine	0.91	9.5	0.83	7.6
Aspartic acid	0.31	3.2	0.50	4.6
Cystine	0.11	1.1	0.15	1.4
Glutamic acid	0.71	7.4	0.76	6.9
Glycine	0.80	8.4	0.88	8.0
Histidine	0.30	3.2	0.39	3.5
Isoleucine	0.48	5.0	0.54	4.9
Leucine	0.70	7.3	0.68	6.2
Lysine	0.56	5.9	0.62	5.6
Methionine	0.34	3.6	0.43	3.9
Phenylalanine	0.44	4.6	0.48	4.4
Proline	0.61	6.4	0.68	6.2
Serine	0.71	7.4	0.77	7.0
Threonine	0.37	3.9	0.39	3.5
Tryptophan	0.36	3.8	0.34	3.1
Tyrosine	0.50	5.2	0.70	6.4
Valine	0.61	6.4	0.63	5.7

^a M. C. Kik, *J. Agr. Food Chem.* **4**, 170 (1956).

^b In air-dried material, 9.8% moisture content.

^c Calculated as grams of amino acid per 16 g. of nitrogen. A conversion factor of 5.95 was used originally, but the data have been recalculated on the basis of the conventional conversion factor of 6.25.

nine and is a little lower in threonine and tryptophan, but these differences may not be great enough to be significant. Compared to soybean oil meal protein, the rice bran and rice polish proteins are significantly higher in arginine, methionine, and tryptophan and, to a lesser degree, higher in histidine and valine.

c. Use in Feeds

(1) *Rice bran*. The high fiber content and low protein level of rice bran limits its use in poultry feeds, although the protein of this feed is

of good quality as shown by amino acid analyses. It has been reported to be satisfactory in chick rations at levels of 10 to 20% of the ration (62).

This feed is used primarily for cattle, being equal to millrun wheat bran in the grain ration for dairy cattle (63, 64). For milk production, rice bran was worth about 75 to 80% of the value of ground corn (64).

Though the high fiber content of rice bran makes it less adaptable to swine feeds than is rice polish, the bran can be used at levels not exceeding 25%. Rice bran is equal to rolled oats and has 90% of the value of corn (64). Above 25% in the feed, there is the danger of producing soft pork because of its high fat content.

(2) *Rice polish*. Rice polish, because of its low fiber content, is more acceptable for poultry feeds but is too low in protein for use in the protein concentrate of a poultry ration. At the 10 to 20% level in chick rations, rice polish has given satisfactory performance (62, 65). For dairy cattle, rice polish has been found to be equal to or slightly superior to corn (64).

Rice polish is best utilized in swine rations, having a feed value the same or superior to that of corn meal (64). Because of its low protein content, rice polish should be supplemented with protein-rich feeds of good quality for satisfactory results (66). Swine feeds should not contain more than 25 to 30% of rice polish; at higher levels, palatability may be affected and the high fat content will produce soft pork.

d. Storage

Rice bran and rice polish, both of which contain high levels of fat, tend to become rancid in storage and should be fed in as fresh a condition as possible. There is available, in some areas, solvent-extracted rice bran which is more stable to storage.

V. SUMMARY

This review includes some of the data available on by-product feeds from the wet milling of yellow corn and sorghum grain and the dry milling of rice. As most feeds today are composed of nutrients obtained from a variety of sources, the data presented combined with the information on other vegetable protein sources may be helpful in the efficient usage of the feeds reviewed.

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CHAPTER 29

MICROBIAL PROTEINS

J. L. STOKES

I. INTRODUCTION

This chapter deals with the nature and distribution of proteins and the related peptides and amino acids in yeasts, molds, mushrooms, and bacteria. Variations in protein and amino acid composition, especially the essential amino acids, as influenced by strain and cultural conditions will be discussed, as well as the advantages and limitations of microorganisms as sources of proteins for animals and humans.

II. YEASTS

1. Composition

The composition of yeasts varies somewhat with the strain and conditions of growth. Representative data for dried brewers' yeast are presented in Table I (1). Total protein is usually calculated by multiplying

TABLE I
PROXIMATE COMPOSITION OF BREWERS' YEAST^a

Component	Content (%)
Crude protein	40-50
Carbohydrate (glycogen)	32-40
Fat	1-2
Crude fiber (cell wall substance)	Up to 10
Ash	6-10

^a P. P. Gray, *Wallerstein Lab. Commun.* **6**, 50 (1943).

total nitrogen by the factor 6.25 on the assumption that the average protein contains 16.0% nitrogen; however, 8 to 13% of the total nitrogen is due to purines, 4% to pyrimidines, 0.5% to choline, 0.5% to glucosamine, and portions to other non-protein constituents (2). There-

fore, only about 80% of the total nitrogen of the yeast cell is in the form of protein.

The yeast cell contains structural, enzyme, and nucleo-proteins. Undoubtedly the bulk of the yeast protein is in the form of enzymes. This is suggested by the knowledge that hundreds of enzymes must be available in the cell for performance of the large number of biochemical reactions involved in energy metabolism and in synthesis. Also, experimental data on crystalline enzymes from yeast indicate that even a single enzyme, phosphoglyceraldehyde dehydrogenase, can account for over 5% of the total yeast protein (3), although most of them range from 0.1 to 0.4%.

Most of our knowledge of yeast proteins stems from the isolation of many enzymes involved in carbohydrate metabolism, in crystalline form, during the past two decades. The old yellow enzyme, alcohol dehydrogenase, glyceraldehyde phosphate dehydrogenase, enolase, hexokinase, phosphoglyceric transphosphorylase, inorganic pyrophosphatase, and transketolase have all been isolated in crystalline form from the cell juice of brewers' or bakers' yeast (4). Others such as carboxylase (5) and lactic dehydrogenase (6) have been only partially purified. The elementary composition of three of the crystalline enzyme proteins was given by Warburg and Christian (7). In all three, the nitrogen content is in excess of 16%, and this emphasizes the difficulty of calculating the exact protein content of yeast or any other biological material from total nitrogen $\times 6.25$.

In addition to proteins, other related nitrogenous compounds occur in yeasts. Nine peptides have been demonstrated by means of paper chromatography in trichloroacetic acid extracts of *Saccharomyces carlsbergensis* (8). Also, as many as sixteen amino acids have been found in the free form within yeast cells, the major ones being alanine, leucine, glutamic acid, and glutamine plus alanine (9). This amino acid pool varies with the growth medium, especially the carbon source, and is also affected by the age of the cells. It enables the yeast cell to synthesize enzyme protein in the absence of exogenous nitrogen and helps explain the unusual ability of yeasts to alter their enzyme patterns. It also accounts for as much as 5 to 12% of the total protein nitrogen of the cells.

2. Amino Acid Composition

Analyses of hydrolyzates reveal that yeast proteins contain a full complement of the usual amino acids. At least, all those that have been tested for have been found. The quantities of the essential amino acids in *Saccharomyces cerevisiae*, the most commonly used commercial

yeast, are given in Table II (10). Additional amino acids in yeasts, calculated to 16% nitrogen, are cystine 1.1%, tyrosine 3.6%, and glutamic acid 14.7% (11). The amino acid composition may vary somewhat with the yeast strain, conditions of growth, and method of assay; but generally these variations do not exceed 10 to 20% (12). The data indicate a close similarity in the amino acid composition of yeast protein with that of some animal proteins, e.g., milk and beef proteins (13).

3. Feed Yeasts

Because they are living cells, yeasts can be expected to contain virtually all the food constituents necessary for the nutrition of other

TABLE II
AMINO ACID COMPOSITION OF *Saccharomyces cerevisiae*^a

Amino acid	Dry weight (%)	Calculated to 16% nitrogen (%)
Histidine	2.7	4.8
Arginine	2.4	4.3
Lysine	3.1	5.5
Leucine	3.8	6.8
Isoleucine	2.5	4.5
Valine	2.8	5.0
Methionine	0.65	1.2
Threonine	2.4	4.3
Phenylalanine	2.1	3.8
Tryptophan	0.59	1.1
Nitrogen	8.94	

^a J. L. Stokes and M. Gunness, *J. Bacteriol.* **52**, 195 (1946).

living systems. Analyses have indicated that this is true. In addition to their high content of protein with a full complement of amino acids, yeasts also contain carbohydrates, fats, minerals, and notably large amounts of B vitamins. Since yeast protein can be produced efficiently, rapidly, and at relatively low cost, the use of yeasts primarily as an inexpensive source of protein in animal feeds has been extensively investigated over many years. The literature on this subject has been frequently reviewed and in recent years by Braude (14), Carter and Phillips (2), Wiley (15), and others (16). Two types of yeasts have been used in feeds. The principal one has been brewers' dried yeast or secondary yeast which is a by-product of the brewery industry. To a much lesser extent primary yeasts such as *Torulopsis utilis* grown specifically for feed purposes have been utilized.

Most investigators have found that dried yeast is an excellent source of protein of high biological value and digestibility for poultry, cattle, sheep, pigs, horses, and other animals. It is especially good for supplying essential amino acids such as lysine which may be present in insufficient amounts in cereal feeds. For example, Macrae *et al.* (17) found that the addition of 5% dried yeast to a maize diet changed it from one unsuited to the rearing of pigs to one which yielded fine animals. Cannon (16) has concluded, on the basis of protein depletion studies with rats, that dried yeast is of excellent protein quality. Although somewhat inferior to milk proteins, it is better than soy protein, sunflower seed protein, peanut protein, navy beans, and peas. When used as the sole source of protein for chickens and rats, yeast protein is intermediate in nutritive value between the high value of good animal proteins and that of inferior vegetable proteins (17). In practical cereal chick rations, yeast protein effectively replaced as much as 80% of animal protein concentrate, but at 100% replacement there was a definite decrease in the growth rate (18). This inadequacy of yeast protein appears to be due largely to its relatively low content of methionine. Thus comparison of six different yeasts with casein to determine their repletion values for protein-deficient adult rats indicated that the yeasts possessed 50 to 85% of the food value of casein. When supplemented with methionine, however, the yeasts gave repletion values equivalent to casein (19). It has also been observed that when yeast is used as the sole source of protein in the diet of rats, liver lesions may sometimes develop (20). But, under practical conditions, feeds contain, in addition to yeast, cereal, oilseed, and animal proteins; the yeast supplies, therefore, only part of the protein of the diet. This eliminates the difficulties which may be encountered when yeast is the sole source of protein in the diet.

Brewers' yeast production has been estimated at 10,000 to 15,000 tons annually in the United States, with a maximum potential of 25,000 tons (15). This limitation does not apply to primary yeasts which can be grown with inorganic nitrogen on a large variety of inexpensive sources of carbohydrate such as molasses, sulfite waste liquors from paper plants, and the wastes of the fruit, vegetable, dairy, and forest industries. A yield of 45 to 50% of dry yeast, containing about 40% true protein, can be expected on the basis of the weight of the sugar fed. These factors are responsible for the continuing interest in yeasts for animal and human food. Also, utilization of industrial wastes for yeast production helps eliminate stream pollution by such wastes. Interest and experimentation with yeast is especially evident in protein-poor countries which usually possess large supplies of carbohydrates and can

obtain the inexpensive inorganic nitrogen which can be converted into nutritious yeast protein. These aspects will be discussed more fully in connection with yeasts for human consumption, but they apply to both feed and food yeast production.

Future expansion of the use of both primary and secondary yeasts in feeds depends primarily on economic factors. In times of emergencies such as both World Wars when economic factors are not paramount, the production of yeast protein expanded tremendously both in the United States and in European countries, especially Germany and England. But under peacetime conditions, yeast protein must compete economically with other plant and animal proteins which are more commonly used in animal and poultry feeds. In this respect, yeast protein has had only limited success so far.

Most operations for the production of primary stock feed yeasts appear to be financially marginal, according to Reiser (21). He calculates that on a production of 9100 pounds of torula yeast per day on the protein waste water from a potato starch plant, the cost of the yeast would be 5 cents per pound. At this price yeast production is not a particularly attractive economic venture, since the competitive products, soybean and meat meal, sell for about the same price. But it would be an important competitor for fish meal, which sells for about 9 cents per pound (1954). Interestingly, Thaysen (22) has pointed out that one acre of land under a carbohydrate crop could yield 840 pounds of yeast protein but only about 70 pounds of meat or milk protein. It seems clear that with the ever-increasing demand for protein concentrates, yeasts offer considerable potential possibilities as relatively inexpensive sources of high-quality protein.

4. Food Yeasts

The advantages and disadvantages of yeasts for feed purposes apply also, in large measure, to their use as a source of protein for human nutrition. During both World Wars Germany produced large amounts of yeast to supplement the existing low-protein diets. It has been estimated that during World War II Germany manufactured food yeast at the high rate of about 100,000 tons annually, primarily from wood sugar solutions (23). During this war there was also some experimentation with food yeast both in England and in the United States (16).

In England a great deal of research was carried out by Thaysen and his associates to develop a commercial process which could supply yeast protein inexpensively to protein-poor areas of the British Empire (22). This work led to the isolation of a new strain, *Torulopsis utilis* var. *thermophila*, which grows well at the temperatures encountered in the

tropics. It is also larger than the usual strains, and this facilitates removal of the cells from the growth medium. Under pilot-plant conditions, the yield of dry yeast with 5% moisture was equal to 60% of the weight of the sugar in the molasses used. The inorganic nitrogen in the medium was virtually completely converted to crude protein. After a growth period of several hours, the yeast is collected, washed, and dried. It comes out as light, straw-colored, thin flakes which have a pleasant nutty or meaty taste. The protein content is 45 to 50%, and the yeast contains the B vitamins. It is readily miscible with water, soups, milk, and stews and can be mixed with flour for the making of bread. Later, a continuous-growth process was described in which one-fourth of the culture is withdrawn every hour and replaced by an equal amount of fresh medium. The yeast population is kept at a level of 2×10^9 cells per milliliter (24). The yeast when dry can be kept for several years without deterioration or loss of its vitamin content.

A commercial plant to produce *T. utilis* from molasses by the Thaysen process was constructed in Jamaica, British West Indies, and is reported to have been in production in 1946. Also, food yeast plants are in operation or planned in South Africa, Formosa, and, in general, in the carbohydrate-rich but protein-poor areas of the Far East. Despite these promising beginnings, there is relatively little use of yeasts at present as a protein food supplement in human nutrition. Thaysen (25) suggests that this situation is due to inertia among food producers, sellers, and consumers; the general public objects to changing its feeding habits. Economic factors are also involved. (See also discussion of food yeast in Chapter 9.)

III. MOLDS

1. Composition

The usual major constituents of living systems—carbohydrates, proteins, lipids, and minerals—are present, of course, in molds. The carbohydrates include reducing sugars, starches, glycogen, hemicelluloses, and also the more difficultly hydrolyzable polysaccharides, cellulosic and lignin-like materials, and chitin; the latter three substances are structural units of the vegetative cell and spore wall. The nitrogen content usually ranges from about 3 to 6% and is, therefore, appreciably lower than the 7 to 10% figures for yeasts. Although most of the nitrogen is present as protein, some of it is in other cell constituents such as chitin, nitrogen-containing lipids, amino sugars, coenzymes, purine and pyrimidine bases, special synthetic products such as penicillin and gliotoxin, and also peptides and amino acids (26).

The nitrogen content, and therefore the protein content, of molds

varies greatly with strain and growth medium. The crude protein content (nitrogen $\times 6.25$) of a number of *Aspergillus* and *Penicillium* species which were grown on a glucose-inorganic salts medium ranged from 13.7 to 43.7% (27). The protein content of these same strains when grown on a glucose-malt sprout medium was considerably lower and ranged from 12.5 to 36.3%. Even greater differences with the two media were found for particular molds. For example, *Aspergillus nidulans* contained 25.6% protein when grown on the inorganic salts medium but only 13.1% when harvested from the malt sprout medium.

Little is known about the specific proteins in molds. An unautolyzable protein has been isolated from *Aspergillus sydowi* (28). The yield was approximately 0.8 g. of protein per kilogram of dry mycelium. It was insoluble in water but soluble in dilute alkali. It contained 11.3% nitrogen and 9.1% reducing sugar and was resistant to hydrolysis by various proteolytic enzymes. A partially purified and identified protein was isolated from cultures of *Penicillium chrysogenum* grown on a glucose-inorganic salts medium (29). It was precipitated from the culture filtrates at pH 3.6 and after purification was found to contain 12.75% nitrogen. Arginine, histidine, cystine, and lysine were identified in the mold protein, and, in general, its composition resembled fairly closely a typical alkali-soluble leaf protein (alfalfa). Another alkali-soluble protein has been isolated from *Aspergillus fischeri* (30). It consisted of two fractions, one of which was insoluble in acid and contained 11.8% nitrogen of which 22.8% was basic nitrogen and 60.2% monoamino nitrogen. The other fraction was soluble in acid and contained 12.3% nitrogen of which 38.0% was basic nitrogen and only 36.5% monoamino nitrogen.

2. Amino Acid Composition

All the essential amino acids have been found in the mycelium of *Rhizopus nigricans*, *Aspergillus niger*, and *Penicillium notatum* (10), and there is no reason to believe that this will not be true for other molds and other amino acids. The specific quantities of the essential amino acids present in the three species of molds mentioned are given in Table III (10). Although *R. nigricans* and *A. niger* have essentially the same quantities of the ten amino acids, *P. notatum* has more of histidine and also of most of the other amino acids. The molds contain roughly only about one-half as much of the amino acids compared to yeasts. This is due largely, although not completely, to the lower protein content of the molds as indicated by their considerably lower nitrogen content.

The mycelium of *A. niger* prior to sporulation, compared to that

TABLE III
AMINO ACID COMPOSITION OF REPRESENTATIVE MOLDS^a

Amino acid	Content of amino acid in:					
	<i>Rhizopus nigricans</i>		<i>Aspergillus niger</i>		<i>Penicillium notatum</i>	
Histidine	0.98 ^b	2.7 ^c	0.90 ^b	2.8 ^c	1.67 ^b	4.4 ^c
Arginine	1.21	3.4	1.04	3.2	1.40	3.7
Lysine	1.59	4.4	1.04	3.2	1.53	4.0
Leucine	1.46	4.0	1.48	4.5	2.1	5.5
Isoleucine	0.98	2.7	0.88	2.7	1.22	3.2
Valine	1.08	3.0	1.09	3.3	1.51	3.9
Methionine	0.33	0.9	0.22	0.7	0.39	1.0
Threonine	0.96	2.7	1.11	3.4	1.37	3.6
Phenylalanine	0.81	2.2	0.85	2.6	1.16	3.0
Tryptophan	0.25	0.7	0.26	0.8	0.48	1.3
Nitrogen	5.80		5.21		6.13	

^a J. L. Stokes and M. Gunness, *J. Bacteriol.* **52**, 195 (1946).

^b Values in this column represent percentage of dry weight.

^c Values in this column represent percentages calculated to 16 g. of nitrogen.

after sporulation, contains considerably larger quantities of most of the essential amino acids largely because of its greater protein content (10). The mycelium and its spores, in general, have comparable amounts of the amino acids. Also, as for yeasts and other microorganisms, the quantities of individual amino acids in molds are constant under constant conditions of growth but may vary considerably when the medium or other cultural conditions are altered.

3. Molds as Animal Food

A limited number of investigations have been made to determine the nutritional characteristics of molds for animals. In rat feeding tests with the dried mycelium of *Penicillium flavo-glaucum*, a normal rate of growth was obtained when the mold was fed at a 9% protein level along with 9% protein in the form of casein or maize gluten (31). These results were interpreted as indicating that the mold mycelium contained sufficient lysine and tryptophan to counteract deficiencies of those two amino acids in maize gluten and also enough cystine to make up for the insufficiency of this amino acid in casein. The amount of cystine even in the mold mycelium, however, is not entirely adequate, since it was the first substance to become limiting in the growth of the rats. When the mycelium was the sole source of protein in the diet, the

rats grew only when the mycelium was fed at an 18% protein level. The proteins of *Aspergillus sydowi* failed to sustain the life of young rats for more than 7 to 9 weeks (32); however, when supplemented, with casein, skim milk protein, egg white, and yeast protein at levels of 2.5 to 5%, the mold proteins supported good growth of the rats. Likewise, the mycelium of *Fusarium lini* B when supplemented with thiamine provides adequate amounts of other B vitamins for normal growth, reproduction, and lactation in rats and compares favorably with brewers' yeast as a protein food (33).

As is true for yeasts, dietary deficiencies of mold proteins due to a low content of sulfur-containing amino acid can be overcome by the addition of small amounts of these amino acids to the mold diet. Thus the addition of 0.25% of either cystine or methionine to otherwise inadequate diets in which several different molds were used as the sole source of protein resulted in good growth of rats (34).

A major difficulty in feeding with molds stems from the low protein content of the mycelium. This makes it necessary to feed the mycelium to the animals in large amounts, especially in those instances in which the mold serves as the sole source of protein. Along with the mold protein, the animal receives large amounts of extraneous mycelial constituents which can produce severe diarrhea. In some instances, at least, this can be avoided by completely autolyzing the mycelium with heat (35).

An interesting situation has been described which concerns the toxicity of *Aspergillus sydowi* mycelium for rats and which may have general implications for mold-feeding (36). The nutritive failure of rats fed on a ration in which all protein and B vitamins were supplied by the mycelium was shown to be due not to any amino acid deficiency but rather to the presence of a toxic substance in the mold which could be removed by absorption on Lloyd's reagent or destroyed by autoclaving or acid hydrolysis.

4. Molds as Human Food

Only a few attempts have been made to supplement human food with mold protein. Most of these experiments took place in Germany during World War II and are described by Robinson (35). Preparations of *Fusarium* and *Rhizopus* as well as several yeasts were fed to human beings. The general health of the people to whom the molds were fed was better than that of those acting as controls; the *Fusarium* strain, which was grown on whey, was the most satisfactory of the organisms tested. The strains of both *Fusarium* and *Rhizopus* were high in cystine, methionine, and glutathione and were therefore especially

suitable for feeding and superior in this respect to the general run of yeast strains. It may, however, be entirely possible to discover also yeast strains with an unusually high content of sulfur-containing amino acids through extensive surveys of many strains of yeasts and cultural conditions.

Another possibility for use of molds as human food is to grow them on soybeans or oilseed press cakes. A product called *tempeh*, formed in this manner, is described in Chapter 9, page 218.

IV. MUSHROOMS

Mushrooms are the fleshy fruiting bodies of the higher fungi or *Basidiomycetes*; they are valued primarily for their unique flavor. It is

TABLE IV
COMPOSITION OF MUSHROOM AND MYCELIUM OF *Agaricus campestris*^a

Component	Percentage of dry weight	
	Mushroom	Mycelium
Crude protein (nitrogen \times 6.25)	37.52	35.5
Fat (ether extract)	1.81	3.3
Nitrogen-free extract (carbohydrates and starches)	38.19	48.8
Fiber	10.38	6.92
Ash	12.00	4.59
Calcium	0.023	0.12
Phosphorus	1.42	1.28
Iron	0.019	Trace

^a H. Humfeld and T. F. Sugihara, *Food Technol.* **3**, 355 (1949).

estimated that about 65 million pounds of mushrooms are grown annually in the United States, and all of them are from one fungus species, *Agaricus campestris*. Other species are cultivated in European countries (37). Although dried mushrooms contain about 40% protein (38) (Table IV), fresh mushrooms, because of their high moisture content, contain only about 3% protein (39). The latter value, however, compares favorably with that of many fresh vegetables. All the essential amino acids and also the B vitamins are present, but the tryptophan content is low. Rats fed on dehydrated mushrooms at the 8% level did not grow nearly as well as with casein or soybean meal. This may have been due, in part, to an insufficiency of some of the essential amino acids in the mushrooms and also to the unpalatability of the mushrooms for the rat.

An interesting recent development has been the production of the

mycelium of *Agaricus campestris* in submerged cultures on fruit and vegetable wastes (38). Under these conditions only the mycelium and not the fruiting body or mushroom is formed. The mycelium, however, has a typical mushroom flavor and can be used as a flavoring material. Its composition closely resembles that of the commercial mushroom (Table IV). The mycelium of another species, *Agaricus blazei*, has been grown successfully under submerged conditions, on orange juice and citrus press water (40). It contained 32.5% protein, on a dry basis, compared to 43% in the fruiting body growing wild. Analyses of hydrolyzates of the mycelium indicated the presence of at least fourteen amino acids. Actually the mycelium of many mushroom fungi can be grown under submerged conditions. Recently twenty species were grown in this way, but only two of them, *A. campestris* and *Lepiota rachodes*, had a pleasant flavor (41). Cultivation of mushroom mycelium offers, therefore, another possibility for converting fruit and vegetable wastes by microorganisms into nutritious protein or desirable flavoring material.

V. BACTERIA

1. Composition

The chemical composition of bacteria, like that of other microorganisms, varies with the species, strain, and cultural conditions. In general, on a dry weight basis, bacteria contain 40 to 80% protein, 10 to 30% carbohydrate, 1 to 30% lipid, and 1 to 14% ash. It is important to note that the protein content of bacteria tends to be greater than that of yeasts and molds.

Part of the nitrogen is due to free amino acids present inside the cells (42). In general, the Gram-positive bacteria are able to assimilate glutamic acid and lysine from the external medium and to concentrate these amino acids in the internal environment, whereas Gram-negative bacteria are unable to do so (43). Other amino acids are also absorbed by bacteria in varying proportions depending on the composition of the medium in which they are growing (44).

Non-dialyzable peptides have been isolated from spores of *Bacillus cereus* and other aerobic spore-forming bacteria but not from the corresponding vegetative cells (45). The spore peptides contained diamino-pimelic acid, glutamic acid, alanine, the acetyl derivatives of glucosamine, and also an unidentified sugar amine.

Although relatively little is known concerning the individual proteins of the bacterial cell, some information has accumulated on the nature of the proteins in the cell wall. Three-quarters of the weight of the cell wall of *Micrococcus pyogenes* is made up of a glycerophospho-

protein complex (46). The protein portion resembles silk fibroin in amino acid composition and in its general properties but differs from silk fibroin in that it contains much less tyrosine and more threonine. This complex is estimated to account for about 20% of the dry weight of the cell. An insoluble protein-carbohydrate complex was isolated from apparently the cell wall of *Corynebacterium diphtheriae* (47). The protein contained glucosamine and a high proportion of diamino-pimelic acid. The oligosaccharide contained two molecules of D-galactose, one of D-mannose, and three of D-arabinose. The cell wall material is apparently composed of 25% carbohydrate, 10% ash, and 60% protein. It is a major component of the entire cell, since it accounts for approximately 40% of the dried cell, on an ash-free basis.

The investigations of Salton (48, 49) have emphasized the variations that can occur in the cell wall composition of bacteria. The cell wall of *Escherichia coli* is lipoprotein, whereas in *Salmonella pullorum*, the cell wall is a lipid-protein-carbohydrate complex. Other bacteria such as *Streptococcus pyogenes*, *Streptococcus faecalis*, *Micrococcus lysodeikticus*, and *Sarcina lutea* have a cell wall which is mucoid in nature and consists of a complex of protein and polysaccharide. A detailed analysis of the material from *Strep. faecalis* indicated that the complex contained about 60% polysaccharide and roughly 25% protein. The former contained glucose, galactose, and rhamnose together with an amino sugar. The protein contained alanine, glutamic acid, lysine, serine, and other amino acids but no aromatic or sulfur-containing amino acids with the possible exception of methionine. The cell wall material equaled 5% of the dry weight of the bacteria. Interestingly, Gram-positive bacteria have a more limited complement of amino acids in the cell wall than Gram-negative bacteria in that they lack aromatic and sulfur amino acids.

An interesting protein which is a growth factor for ameboid slime molds has been isolated from three Gram-negative bacteria (50). It was extracted with dilute alkali from cells ground with alumina and represents 6% of the dry weight of the bacteria. The protein cannot be isolated from cells disrupted by cytolysis with glucose or toluene which suggests that these treatments inactivate or solubilize it.

2. Amino Acid Composition

Analyses of hydrolyzates of representative bacteria show that the essential amino acids are present and in amounts which are similar to those in most plant and animal proteins (Table V). Marked differences occur among the bacteria. *Escherichia coli*, on a dry weight basis, contains approximately twice as much histidine, arginine, leucine, valine,

TABLE V
AMINO ACID COMPOSITION OF SOME REPRESENTATIVE BACTERIA^a

Amino acid	Content of amino acid in:					
	<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>		<i>Bacillus subtilis</i>	
Histidine	0.72 ^b	1.1 ^c	1.26 ^b	1.5 ^c	0.87 ^b	1.4 ^a
Arginine	2.3	3.4	4.3	5.2	2.4	3.8
Lysine	5.2	7.8	4.5	5.5	3.4	5.4
Leucine	3.4	5.1	6.4	7.8	4.8	7.6
Isoleucine	2.8	4.2	3.8	4.6	3.0	4.8
Valine	2.4	3.6	4.5	5.5	3.5	5.6
Methionine	0.81	1.2	1.7	2.1	1.08	1.7
Threonine	2.0	3.0	3.2	3.9	2.2	3.5
Phenylalanine	1.84	2.7	2.7	3.3	2.2	3.5
Tryptophan	0.23	0.34	0.79	0.96	0.38	0.6
Nitrogen	10.75		13.19		10.07	

^a J. L. Stokes and M. Gunness, *J. Bacteriol.* **52**, 195 (1946).
^b Values in this column represent percentage of dry weight.
^c Values in this column represent percentage calculated to 16 g. of nitrogen.

methionine, and phenylalanine and three times as much tryptophan as *Staphylococcus aureus*. The latter exceeds *E. coli* only in having a slightly greater amount of lysine. *Bacillus subtilis* is intermediate between *S. aureus* and *E. coli* with respect to content of the ten amino acids. Analyses of other bacteria give results that are, on the whole, similar to those reported above (51, 52); these investigations included several amino acids in addition to the essential ones.

In contrast to the fluctuations of internal free amino acids with variations in the growth medium, the amounts of amino acids found in proteins remains relatively constant (42, 44). Small variations in the over-all amino acid composition of bacteria can occur with changes in cultural conditions, but these are usually of the order of 10 to 20%, although occasionally they may be as much as 100% (10, 52). Even small changes in amino acid composition, however, may represent major changes in specific proteins, for example, in enzyme proteins.

3. Bacteria as Food

A process has been developed for producing dried cells of *Bacillus megaterium* primarily for use as a vitamin B₁₂ concentrate, but the results indicate that the cells also may have nutritional value as a source of protein (52). The cells are grown for 6 hours with aeration on a molasses-inorganic salts medium; approximately 50% of the

weight of the sugar is converted to bacterial solids. The cells are harvested by centrifugation and are drum-dried to give a pale-tan, free-flowing, non-hygroscopic product which possesses most of the protein and vitamin nutritional values of yeast in addition to containing 15 mg. of cobalamin (vitamin B₁₂) per kilogram. No toxic effects were observed in rats fed on diets containing up to 20% of the dried *B. megaterium* cells for 400 days (53). These results have suggested that *B. megaterium* can be used safely in human and animal nutrition and that it could be a satisfactory substitute for yeast in the conversion of molasses into protein- and vitamin-rich supplements.

There is some indication that cultures of coliform bacteria can increase the growth rate of chicks and poults (54, 55). Also, unidentified dried bacteria obtained from bovine rumen fluid were found to have essentially the same biological value and digestibility as yeast (56).

VI. SUMMARY AND CONCLUSIONS

Microbial cells contain free amino acids and peptides in addition to proteins. In so far as can be judged from nitrogen determinations, bacteria in general contain more protein than yeasts, and the latter more than molds. Relatively little is known about the nature of the specific proteins in microorganisms. Most of the available information on yeasts concerns several isolated crystalline enzyme proteins which participate in the dissimilation of carbohydrates. In the case of bacteria, information is accumulating on the nature of the proteins in the cell wall and also the lipid and carbohydrate components with which the proteins are closely associated.

Analyses indicate the presence of all the essential amino acids in microorganisms, and the available evidence is compatible with the generalization that the proteins of bacteria, yeasts, and molds contain a full complement of amino acids. Conversely, there is no evidence, with the newer and more accurate and specific microbiological and chemical methods, that any of the amino acids is entirely absent from any microorganism.

The quantities of the individual amino acids in the proteins of different microorganisms may vary by as much as 100% or more, but there are no consistent differences between bacteria, yeasts, and molds as groups. Also, the amino acid composition of a microorganism can vary with changes in the composition of the growth medium and other environmental conditions. When such changes occur, the major variation appears to be in the free amino acids rather than in the proteins. The latter, however, probably also change to some extent as the enzymatic composition of the cell varies.

There are no clear-cut major differences between microbial proteins and the proteins of other plants and of animals. Variations within each group are as great or greater than variations between groups. Because of these basic similarities among all living systems, it could be predicted that microbial proteins would be readily utilized by animals and humans and the extensive data which have accumulated fully support this prediction. The excellent nutritional value of yeasts as sources of protein has been repeatedly established. The molds and bacteria have been studied to a more limited extent. But even on the basis of these few investigations, there is reason to believe that molds and especially bacteria will be suitable for feeding purposes. The bacteria in particular merit further investigation because of their high protein content and their content of vitamin B₁₂.

The advantages of microorganisms as sources of protein and other nutritional factors are their rapid growth, their great synthetic abilities which enable them to convert efficiently waste carbohydrates and inorganic nitrogen into high-quality protein, and the ease with which they can be controlled and modified through selection of strain and cultural conditions. Although microbial proteins are in only limited use at present as animal and human food, they remain very important potential food sources. Microbial proteins will undoubtedly come into greater use as inertia to change in habits of food production and consumption decreases and as economic factors become more favorable for competition of microbial proteins with conventional plant and animal protein foods.

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CHAPTER 30

THE ALGAE

W. A. P. BLACK

I. CLASSIFICATION

The basic issue in livestock feeding in most parts of the world is to provide protein supplements for cheap available energy sources. In their quest for available sources of proteins, many countries have turned to the algae which, since the earliest times, have been used by both man and animal as a source of food.

Consisting of over 17,000 species, the algae belong to the lowest division of plant life (Thallophyta) and are usually grouped into eleven main classes, or classified according to their predominant pigment, into blue-green algae (Myxophyceae), green algae (Chlorophyceae), red algae (Rhodophyceae), and brown algae (Phaeophyceae). For detailed treatment of their morphology the reader is referred to Fritsch (1) or Smith (2).

II. HISTORICAL BACKGROUND

Although an extensive literature is available in which the gross chemical composition of many of the algae is recorded, the amino acid composition of the algal proteins has received little attention, and little is known of the type of proteins present. A review by the present author (3) summarizes the work in this field. Recent work (4, 5) on the free amino acids, iodoamino acids, peptides, and isolated proteins of a number of algae has shown that the results of earlier workers were erroneous (6) and that the protein amino acids of various algae are qualitatively similar and differ little from those of land plants. Fowden (7) has also isolated protein fractions from members of four different algal classes, *Chlorella vulgaris*, *Anabaena cylindrica*, *Navicula pelliculosa*, and *Tribonema aequale*, compared them with those of other plant proteins, and found a close similarity in their amino acid compositions. A complete quantitative analysis of the proteins of *Chlorella vulgaris* has also been reported (8) and the amino acid composition shown to re-

main virtually constant with increasing culture age, although the percentage of protein within the cells decreased (9). Other work in this field is reviewed by Fowden (7) and Fogg (10), who discuss the limitations of our present knowledge and point out that the amino acid composition of the algal proteins may give no information regarding the nature of the individual proteins and the arrangement of the amino acid residues within the molecule and that proteins with similar amino acid composition could have quite different biological activity.

In Japan, also, attention has been paid to the nitrogenous constituents of the algae (11, 12), and Takagi has determined the amino acids in *Ulva pertusa* and *Enteromorpha linza* (13) and studied the protein of *Ulva pertusa* (31.55% of the dry matter) (14). He also determined tyrosine, tryptophan, threonine, and serine (15) in over thirty species and concluded that the brown and red algae were as rich as other plant or animal proteins in tyrosine and serine.

Doubt exists as to the occurrence of the iodoamino acids. Roche and Lafon (16) originally reported 3,5-diiodotyrosine, but conflicting evidence has arisen (5), and work on iodoamino acid stability and iodine lability under conditions of barium hydroxide hydrolysis, in which I^{131} was used, seems to indicate that all the iodoamino acids except thyroxine are unstable and the iodine labile (17).

III. MICROSCOPIC ALGAE

1. Plankton

The portion of the sea wherein there is sufficient light penetration to support attached plants constitutes only about 2% of the sea floor, and consequently the material produced by the attached marine plants (seaweeds) is relatively small, and the primary food production becomes mainly a function of the unattached floating plants, notably the phytoplankton which, though microscopic in size, occur in vast incalculable quantities. It has frequently been suggested that marine plankton could be used to supplement our food reserves. (See also Chapter 9.) Clarke and Bishop (18) studied the nutritive value of marine plankton with a consideration of its use as an emergency food. The material, however, was mainly zooplankton retained by a coarse plankton net. The dry plankton was composed of 52 to 59% protein, 1 to 4% fat, 13 to 17% carbohydrate, and 19 to 33% ash. When fed as a third of the rat's diet the material had some nutritive value, but when fed as their sole diet the rats lost weight and died within 4 to 19 days.

The harvesting of plankton has been examined from engineering and economic viewpoints (19). It was concluded that plankton har-

vesting on a large scale would not be economically feasible unless areas of greatly increased population ratios were located or produced artificially, or until a radically novel and cheaper method of harvesting was evolved. In addition, it was pointed out that not all forms of plankton are palatable or even nutritious, and some are even toxic and would present serious problems in bulk harvesting. In the sea the fish collect the plankton, and, as the quantity of fish harvested is still statistically negligible compared with their availability, it would appear to be more logical to allow the fish to harvest the plankton and increase our fish catches.

2. Cultured Unicellular Algae

a. Mass Culture

World-wide interest in the mass culture of algae has resulted in an extensive literature which has been summarized in "Algal Culture" (20) and recent reviews (21-21b). Most of the experiments have been carried out with species of *Chlorella* which grow rapidly, tolerate a variety of cultural conditions, and have a variable composition which can be altered at will by careful control of the nutrient solution, the light intensity, and the age of the culture. The main engineering problems of providing means for the algae to grow continuously at the maximum rate and of harvesting some of them without disturbing the growth of the remainder have been studied in a number of countries (20), and pilot plants have been constructed. On the experimental scale, harvesting is usually by high-speed centrifuging, resulting in a paste containing 75% of water, and this is then dried by one of the conventional methods such as spray drying. The problem of large-scale separation, however, is as yet unsolved, as the cost of centrifuging would probably be prohibitive. The dried cells can contain up to 88% of proteins, vitamins, and all the essential amino acids required of a foodstuff (22-25).

b. Composition, Digestibility and Utilization

The elementary composition of a number of unicellular algae, marine algae, and leaves of land plants has been determined by Milner (26), and the proteins have been examined by Fowden (7). Table I, compiled by Fogg (10), gives the amino acid composition of the bulk proteins of various algae compared with similar data for higher plants.

Chlorella has been fed to rats and chicks (27). When 10% was added to the basal chick ration in place of soybean meal, a marked increase in growth and improvement in feed efficiency resulted, the algae

TABLE I
THE AMINO ACID COMPOSITION OF THE BULK PROTEINS OF VARIOUS ALGAE COMPARED WITH SIMILAR DATA FOR HIGHER PLANTS^a
(calculated as grams of amino acid nitrogen per 100 grams protein nitrogen)

Amino acid	<i>Chlorella vulgaris</i>	<i>Ulva</i> sp.	<i>Fucus</i> ^b <i>vesiculosus</i>	<i>Navicula pelliculosa</i>	<i>Laminaria</i> sp.	<i>Chondrus</i> sp.	<i>Microcystis aeruginosa</i>	<i>Anabaena cylindrica</i>	<i>Phormidium</i> sp.	Higher plants, Gramineae ^c
Aspartic acid	6.4	4.1	9.0	6.4	1.9	2.5	4.6	6.9	0.9	4.9-5.4
Glutamic acid	7.8	7.6	11.2	4.9	7.3	8.2	6.5	5.6	4.4	6.6-7.8
Serine	3.3	—	3.5	4.2	—	—	3.3	2.4	—	—
Threonine	2.9	—	3.3	4.2	—	—	3.2	5.7	—	3.0
Glycine	6.2	0.8	5.4	6.1	2.7	2.1	4.9	5.5	1.6	0.4
Alanine	7.7	6.5	5.4	6.5	6.4	3.7	5.4	6.0	5.2	4.4-5.1
Valine	5.5	5.2	3.0	7.5	5.1	2.8	4.1	7.0	6.7	3.3-4.2
Leucine	6.1	5.2	5.0	7.2	2.5	5.3	4.2	6.2	2.1	7.1-8.8
Isoleucine	3.5	—	3.0	3.5	—	—	2.2	3.9	—	—
Phenylalanine	2.8	2.3	2.6	3.4	1.0	1.5	4.4	2.9	1.1	2.5-2.6
Tyrosine	2.8	0.0 ^d	1.2	1.9	1.9	2.3	—	1.6	1.8	2.3-2.5
Proline	7.2	7.0	3.3	6.2	7.6	7.1	3.2	5.0	7.0	3.1
Tryptophan	2.1	0.3	—	1.1	1.1	1.6	—	1.0	0.2	1.8-2.1
Methionine	1.4	0.0 ^d	0.4	1.2	0.0 ^d	0.0 ^d	1.7	1.2	2.0	1.4-1.6
Cystine	0.2	1.8	—	—	3.4	1.6	—	—	0.0 ^d	1.3-1.5
Arginine	15.8	7.5	9.4	9.2	16.1	10.2	—	11.7	9.2	13.7-14.3
Histidine	3.3	1.2	1.6	2.8	1.6	1.8	—	2.5	3.8	3.6-3.7
Lysine	10.2	0.0 ^d	6.0	8.3	0.0	4.0	—	6.6	0.0 ^d	6.3-6.6
Amide-N	6.1	—	—	7.1	—	—	—	8.0	—	4.7-5.3

^a According to G. E. Fogg, "The Metabolism of Algae," Methuen, London, 1953.
^b D. G. Smith and E. G. Young, *J. Biol. Chem.* **205**, 849 (1953).
^c Bulk protein of leaves. J. W. H. Lugg, *Advances in Protein Chem.* **5**, 237 (1949).
^d Not detected by the methods used. A. Mazur and H. T. Clarke, *J. Biol. Chem.* **123**, 729 (1938).

providing important quantities of carotene and certain B-complex vitamins. Rats, on the other hand, did not do so well unless the cellulose cell wall was broken down. If this was not done, the rats failed to grow, being unable to digest the cell wall and obtain the proteins. It is doubtful, therefore, if *Chlorella* will ever provide a direct source of food for man, as rats behave rather like man as far as protein is concerned. A mixture of unicellular algae has, however, been fed to lepers (28), and the daily ingestion of 20 grams for two years produced an increase in weight and an improvement in general health; no toxic effects were observed.

Preliminary experiments have been carried out in Japan on the use of *Chlorella* as human food (24). The algal cells, separated from the culture medium, were washed with water by centrifuging, the thick paste dried at room temperature with infrared lamps, and the dried mass ground to a fine green powder. The composition of the cells was estimated as 42% protein, 22% fat, 24% carbohydrate, and 12% ash; qualitative assays were carried out for the amino acids and sugars, and quantitative assays for vitamins A (5000 I.U./g.), B₁ (4 γ /g.), B₂ (21 to 27 γ /g.), B₆ (9 γ /g.), niacin (176 γ /g.), folic acid (485 γ /g.), and vitamin C (2000 to 5000 γ /g.). The dried *Chlorella* cells were found to have a taste and flavor similar to that of powdered green tea and could be added to various kinds of food, both Western and Japanese, giving them an agreeable taste and appearance and increasing their fat, protein, and vitamin content.

Promising results have been obtained by Fink (28a), who, for a period of three years, fed rats with twelve different samples of the alga *Scenedesmus obliquus*, cultured at the Casbon Biological Research Station, Essen, and dried by infrared. The dried alga seemed to stimulate the appetite of the young rats, and in all the trials the rats on the algal diet ate more. Fink concluded that the biological value of the protein of this alga was equal to that of the protein of dried skimmed milk and superior to any protein of plant origin so far examined by him. In addition, when the alga contributed 92% of the animal's protein requirements no supplementation by cereal protein was necessary as was the case with dried skim milk. The dried alga appeared to contain one or more substances which singly or together were able to prevent dietetic liver necrosis.

c. Cost of Production

Despite the work which has already been carried out, there is still no sound basis for estimating the cost of production on a commercial scale. The feasibility of this method of producing a palatable and nu-

tritional foodstuff, high in proteins, has been amply demonstrated, and the existing evidence indicates that algal farms in the tropics might yield 20 tons (dry weight) of food per acre per year, with higher yields if the air was enriched with carbon dioxide. Milner (23) estimates that *Chlorella* could be mass-produced at 25 to 30 cents a pound (20 tons per acre), but on the basis of cost per pound of protein this is out of line with the present cost of high-protein foods. If the yield could be doubled and the cost halved, *Chlorella* would then become highly competitive with farm produce. It seems inevitable, however, that algal culture will occupy an important place in future world food production, particularly in countries possessing ample water and sunshine but poor soil, and in areas of the world where famine is ever present.

IV. MACROSCOPIC ALGAE

The multicellular or macroscopic algae which are fixed to the sea bottom and are generally referred to as seaweeds, or kelp in America, are confined to inshore waters at a depth to which light can penetrate. They are composed chiefly of the red, brown, and green algae, but the latter do not occur in sufficient quantity to justify their consideration as a source of protein.

1. Red Algae

Very little is known about the location and potential availability of the red algae, the bulk of which grows sublittorally. Little progress can be made until methods of locating and assaying them have been developed. From such statistics as are available, at least 50,000 tons of wet red algae are harvested annually from the shores of fifteen maritime countries for subsequent conversion into human food, animal feedstuffs, and chemicals. These algae consist of four main classes: (1) agar-producing weeds such as *Gelidium* spp., *Gracilaria confervoides*, *Furcellaria fastigiata*, and *Ahnfeltia* spp., used in foodstuffs and for the preparation of agar for food and bacteriological purposes; (2) carrageenin weeds such as *Gigartina* spp. and *Chondrus crispus*, used in the preparation of carrageenin for foodstuffs; (3) *Rhodomenia palmata*, used directly in foodstuffs; and (4) *Porphyra* spp., also used in foodstuffs.

In view of the popularity of *Porphyra* (laver) as a foodstuff in the Far East (3000 tons dry matter are produced in Japan per annum), the nitrogen distribution in it has been investigated (11). Samples of different species from different habitats at different times of the year were studied, and the nitrogen distribution correlated with the seasons. Di-

gestibility trials were carried out with human beings when the *Porphyra* (crude protein 33.1%) was fed in conjunction with rice, and digestibility figures of 72.6, 14.6, 75.2, and 25.6% were obtained for protein, fat, carbohydrate, and fiber, respectively (29).

The biological value of the proteins in *Rhododymenia palmata* (dulse) has been determined and found to be 42% (30). This is the species se-

TABLE II
COMPOSITION OF THE DRY MATTER OF VARIOUS RED ALGAE^a

Species	Source	Crude protein (N × 6.25) (%)	Total ash (%)	Fats and pig- ments ^b (%)	Cellu- lose (%)	Reducing sugars (anhydro sugar) (%)
<i>Rhododymenia palmata</i>	North Berwick, Scotland (6/11/52)	21.9	21.2	2.4	2.4	36.2
<i>Ahnfeltia</i> spp.	Plymouth, England (4/11/52)	24.4	25.6	0.7	8.8	41.4
<i>Gelidium pristoides</i>	Strandfontein, South Africa (22/6/52)	21.3	19.8	1.0	6.5	38.7
<i>Gracilaria confervoides</i>	Langebaan, South Africa (10/6/52)	23.8	31.0	1.1	3.8	32.0
<i>Ptilota plumosa</i>	Dunbar, Scotland (24/4/52)	25.6	34.4	1.2	4.7	30.0
<i>Porphyra umbilicalis</i>	North Berwick (24/4/52)	27.5	21.8	3.2	3.2	43.2
<i>Dilsea edulis</i>	Plymouth (3/11/52)	19.3	19.8	0.8	3.1	45.4
<i>Polysiphonia fastigiata</i>	North Berwick (4/3/52)	30.0	24.9	2.2	1.1	35.6
<i>Gigartina stellata</i>	North Berwick (30/1/52)	22.5	21.2	1.6	2.3	38.9
<i>Chondrus crispus</i>	Aberayron, Wales (22/2/52)	19.3	20.8	1.2	2.0	39.3

^a According to A. G. Ross, *J. Sci. Food Agr.* **4**, 333 (1953).

^b Soluble in carbon tetrachloride.

lectively chosen by the sheep in Orkney where the sheep live almost entirely on seaweed (31).

A proximate analysis, carried out by Ross (32), of the red algae common to Britain is given in Table II; the analyses of other red algae are included in Tables III to V.

Chemical analysis indicates, therefore, that the red algae are a comparatively good source of proteins (20 to 40% of dry matter) of high biological value, containing all the essential amino acids, and, in addition, are a valuable source of vitamins and minerals; but until

TABLE III
COMPOSITION OF DRY MATTER OF VARIOUS ALGAE COMMONLY USED AS FOOD IN JAPAN^a

Species	Crude protein (%)	Fat (%)	Carbohydrates		Ash (%)	Vitamins				
			Sugar (%)	Fiber (%)		Provitamin A (I.U./g.)	B ₁ (γ/g.)	B ₂ (γ/g.)	Niacin (γ/g.)	C (γ/g.)
<i>Phyllocladus sacrum</i> (Cy) ^{b,c}	26.9	0.1	64.4	1.3	7.5	4.9	2.4	4.0	—	—
<i>Prasiola japonica</i> (Ch) ^b	42.0	1.8	46.0	5.6	4.7	2.8	5.2	3.8	—	—
<i>Enteromorpha compressa</i> (Ch)	21.5	0.3	64.0	7.5	6.9	30.0	0.6	3.1	80.3	100
<i>Monostroma nitidum</i> (Ch)	16.0	0.2	66.6	5.3	12.1	—	—	—	—	—
<i>Laminaria japonica</i> (Ph)	8.6	1.3	60.9	3.5	25.8	5.2	0.9	3.8	21.1	130
<i>Laminaria angustata</i> (Ph)	8.2	2.0	60.0	6.6	23.5	—	0.25	2.4	—	—
<i>Laminaria religiosa</i> (Ph)	7.2	0.4	49.7	8.0	35.0	0.11	0.84	2.3	—	—
<i>Undaria pinnatifida</i> (Ph)	15.1	1.8	57.0	4.3	22.0	5.3	1.3	1.7	120	180
<i>Hijikia fusiformis</i> (Ph)	6.7	0.96	35.8	15.6	40.9	5.4	0.12	2.4	48	0
<i>Fisenia bicyclis</i> (Ph)	9.3	0.1	63.2	12.2	15.5	1.9	0.25	2.5	—	—
<i>Nemacystus decipiens</i> (Ph)	2.7	1.5	2.3	—	93.7	—	1.5	1.5	—	—
<i>Heterochordaria abietina</i> (Ph)	22.4	5.1	46.5	6.3	19.9	—	—	—	—	—
<i>Gracilaria confervoides</i> (Rh)	14.0	1.2	66.7	3.0	15.2	48.5	0	1.8	—	0
<i>Porphyra tenera</i> (Rh)	40.2	0.8	44.7	5.3	9.0	445	2.8	14.0	110	230

^a From Standard Tables of Food Composition in Japan, edited by the Committee on Food Composition, Resources Council, Prime Minister's Office, Japan, 1954.

^b These are algae growing in fresh water, others being marine algae.

^c Abbreviations: (Cy)—*Cyanophyceae*; (Ch)—*Chlorophyceae*; (Ph)—*Phaeophyceae*; (Rh)—*Rhodophyceae*.

surveys are carried out no estimate can be given of their potential availability.

2. Brown Algae

a. Availability

The brown algae can be conveniently divided into two types: (1) the littoral weeds, which occur on the shore between high and low water and which can only be harvested manually giving *Ascophyllum*



FIG. 1. Bed of *Laminaria* exposed at low tide, North Berwick, Scotland.

meal and (2) the sublittoral weeds which grow below water and which give rise to *Laminaria* meal (*Laminaria* spp.) (Fig. 1) or kelp meal (*Macrocystis* spp.). Until recently, few attempts have been made to survey the seaweed-bearing coasts of maritime countries, partly because of lack of interest and partly on account of the difficulty of assessing accurately the nonbuoyant types. Since 1945, however, a number of detailed surveys have been carried out on a reasonably sound scientific basis, but our knowledge of the world's seaweed resources is still strictly limited and is often based on observations of doubtful value. The first coordinated effort to define the areas supporting brown and red seaweeds in sufficient quantity for commercial exploitation was

made in Edinburgh in 1952, and the information has been recorded in two maps (33).

Less than ten accurate quantitative surveys of areas supporting brown seaweeds have been undertaken and reported in the literature.

KNOWN SUBLITTORAL BROWN SEAWEED RESOURCES

Area	Wet weight (million tons)
United States (34)	
Alaska	19.2
Puget Sound, San Diego	13.8
Mexico (34)	
San Diego-Cedros Islands	8.5
Canada	
British Columbia (35)	0.32
Southwest Nova Scotia (36)	0.9
Europe	
Norway (37)	About 20
Scotland (38)	About 10
Russia (White Sea) (39)	1.5
Australasia	
New Zealand (40)	0.8
Tasmania (41)	0.35
Sargasso Sea (42)	4-11

In many cases the 40-year-old estimates are open to question, and, although the coastlines of, for example, south Peru, Chile, southern Argentina, Falkland Islands, and western South Africa are all fringed with extensive beds of weed, no reliable estimates have been made in these areas. Accurate methods of surveying, based on aerial photography and spring grab technique, have recently been worked out and are currently used in Scotland, Norway, and Nova Scotia. Until such methods are universally adopted, no approximate estimate of the world's brown seaweed resources can be given, but the figures for Scotland and Norway, namely 10 and 20 million tons, respectively, indicate that vast quantities are available for commercial exploitation. Virtually nothing is known about the extent to which a seaweed crop in any particular area varies with the season and from one year to another; preliminary studies in Scotland have revealed that these variations are significant.

b. Factors Affecting the Protein Content

Considerable work has been carried out in France (43, 44), Norway (45), Canada (46), Japan (47), and Britain (48) on factors af-

fecting the chemical composition of the brown algae. The chemical composition depends on species, season of the year, habitat, depth at which the alga grows, and stage of development (48). Concentration gradients are found in both the *Fucaceae* (littoral weeds) (49) and

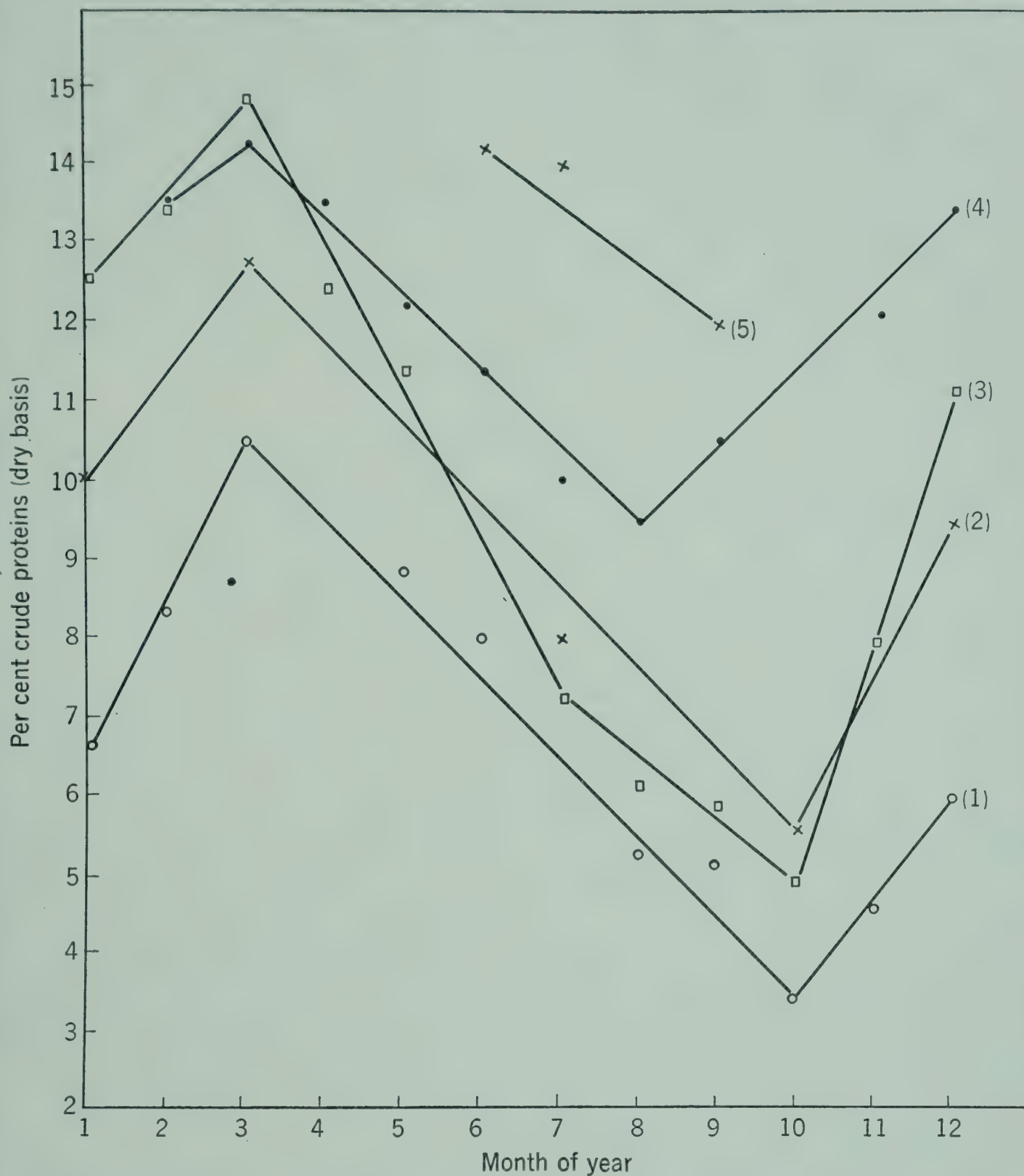


FIG. 2. Seasonal variation in crude protein content of the brown algae common to Scotland. (1) *Ascophyllum nodosum* (Oban), (2) *Laminaria digitata* frond (Loch Melfort), (3) *L. saccharina* frond (Shuna Island), (4) *L. cloustoni* frond (Cullipool), (5) *L. digitata* frond (Inchcolm, Firth of Forth).

the *Laminariaceae* (sublittoral weeds) (50). In Fig. 2, the seasonal variation in the crude protein content is given for the common rock-weed, *Ascophyllum nodosum*, and the most predominant Scottish sublittoral weeds, *Laminaria cloustoni*, *L. digitata*, and *L. saccharina*. As

with land crops, the protein is at a maximum in the spring and decreases as growth proceeds and as the nutrients, particularly nitrate, in the seawater are reduced by being utilized. A correlation has been shown to exist between the chemical composition of the algae and the composition of the seawater in which they grow. Considerable variation occurs, therefore, in the crude protein of the same species from different habitats. In Fig. 2 it can be seen that the crude protein in *L. digitata* frond from Inchcolm is consistently 6% higher than at Loch Melfort, owing to the enrichment of the water at the former habitat with nitrate as a result of sewage disposal. Considerable variation occurs also in the protein content in different parts of the same alga (50). The transition zone of the *Laminarias*, which is the active growing part, is generally lowest in protein nitrogen (15%) and high in free amino acids; two-thirds of the way up the frond the protein appears to be at a maximum (20%) (50).

As with other plant material, considerable changes can occur in the protein content in the period which elapses between harvesting and drying. The ensiling of algae has been studied both on the laboratory and on the pilot scale (51), and it has been found that they support a vigorous lactic acid fermentation and can be ensiled without inoculation or the addition of fermentable carbohydrates. The chemical changes that occur on ensiling depend on the initial composition of the material, and the extent of the change, therefore, depends on the time of year at which the algae are harvested. With *Ascophyllum nodosum*, very little change in composition occurs with season, and preservation can be accomplished by ensiling, the only significant change being an increase in non-protein nitrogen at the expense of protein nitrogen. With *Laminaria cloustoni*, however, if harvested in the spring of the year when inorganic nitrogen is present, ensiling results in protein synthesis with utilization of mannitol. If harvested later in the year, when laminarin is present and inorganic nitrogen is absent, fermentation results in the utilization of the laminarin and a breakdown of proteins.

c. Production of Seaweed Meals

The algae, when harvested, rapidly undergo bacterial decomposition, so that for storage purposes they have to be dried as soon as possible after harvesting. Ensiling offers a means of preserving the fresh weed, and in Ireland seaweed is often ensiled with hay and fed to livestock during the winter months. Seaweed meals can be divided into two main types: (1) *Ascophyllum* meal, consisting of the rockweed *Ascophyllum nodosum*, and (2) *Laminaria* meal, from *Laminaria cloustoni* or one of the other sublittoral weeds.

The littoral weeds which grow on the rocks between high and low water are collected manually, and, with a serrated hand sickle, it is possible for one man to collect 15 cwt. of fresh *Ascophyllum nodosum* per hour. The cut weed is then either forked directly into transport and conveyed to the dryer, or, alternatively, it can be put into nets which can be towed in at high water. No mechanical method has yet been devised for collecting this type of weed. The weed (moisture content 75%) if spread out on drying racks can be air-dried to a state suitable for milling (20 to 25% moisture) in 48 hours or less. In Scotland, where the humidity does not permit air-drying for any length of time, the weed is dried in a kiln dryer or in a conventional continuous-belt conveyor type of dryer. In the latter dryer the fresh weed is subjected in the first stage to a blast of hot gases (about 300°C.) for about 5 minutes and in the second stage to air at approximately 170°C. for about 15 minutes. The weed (moisture content 15 to 20%) leaving the dryer is then conveyed to a conventional hammer mill fitted with a 1/4-inch screen. The resulting product is a green or greenish-brown hygroscopic powder with a characteristic odor. It is usually stored in sacks and at reasonable humidities (<75%) is quite stable.

The harvesting of the sublittoral weed is a more difficult problem on which considerable research work has been carried out. In the southern hemisphere the giant kelp (*Macrocystis* spp.) is harvested by means of a boat fitted with reciprocating cutting gear which, by law, is only allowed to cut to a depth of 4 feet. The cut weed which floats on the surface is then collected in nets and is later dried in a rotary kiln dryer to give the kelp meal. In Canada, Norway, and France, where mainly *Laminaria digitata* is harvested, this is either done manually by cutting the exposed weeds at low water or by means of a small grapnel which is thrown overboard and dragged along the sea bottom. Work has been carried out at the Institute of Seaweed Research, Scotland, on the mechanization of an underwater seaweed harvester, and the feasibility of using a continuous-belt harvester (52) has been proved. Harvesting of this type of sublittoral weed (*Laminaria* spp.) is more difficult, as the weeds grow at depths down to 12 fathoms on an uneven rocky bottom and are heavier than water; consequently, a collecting device has to be incorporated with any type of cutting mechanism. In Norway the weed is usually collected during the summer months and air-dried. In Scotland only the cast stipes of the sublittoral weeds (*Laminariaceae*) are air-dried and serve as starting material for the alginate industry. Any future large-scale development in Scotland will no doubt depend on the harvesting and utilization of this sublittoral weed. Atmospheric conditions are unsuitable for the

natural drying of the fronds, and consequently research work is in progress to find the most suitable type of dryer. Pulping the fresh weed in a hammer type of mill and the drying of this pulp on a drum dryer may prove to be a suitable method.

d. Composition of Seaweed Meals

The composition of the main types of seaweed meal is given in Tables IV and V. Like land plants, seaweeds are composed mainly of carbohydrates, fats, proteins, and mineral matter, but they are also rich sources of vitamins and growth-promoting factors. Seaweeds have the advantage over land crops in that they grow in an ideal environment in which the nutrients are being constantly renewed by nature and they have the remarkable power of concentrating several thousand times many of the elements found in seawater (53).

The main carbohydrates found in the brown seaweeds are mannitol, laminarin, fucoidin, alginic acid, and cellulose. The sweet-tasting mannitol takes the place of the sugars of the land plants. In small amounts it is readily utilized and may be partly converted to glycogen, but in large doses it can have a pronounced laxative effect. The fate of mannitol in man and animals has been studied by a number of workers, and their work has been reviewed (54). In place of the starch of the land plants, the brown seaweeds contain laminarin (55, 56), a glucose polymer which behaves toward enzymes in much the same way as starch and which is as readily utilized as maltose by the bacteria in the bovine rumen. No metabolic studies have been carried out with fucoidin (57), but feeding trials have shown it to have a laxative effect. Alginic acid (58), the main cell-wall constituent of the brown seaweeds and bearing some relation to vegetable pectin, has, in some animal and human experiments, been shown to have considerable nutritive value (59, 60).

The cellulose content of the brown seaweeds is low compared with that of land plants (61), but roughage in a diet containing seaweed would be provided by fucoidin and alginic acid, carbohydrates which are probably not utilized but which are capable of absorbing considerable quantities of water. Varying amounts of fats are present from 1% in the *Laminaria* to 8% in the most exposed weed, *Pelvetia canaliculata* (62).

Seaweeds contain all the elements present in seawater and, therefore, all the elements (Table V) which have so far been shown to play an important part in the physiological processes of the animal. They cannot be regarded as a complete, balanced mineral supplement, being low in calcium and phosphorus; but these deficiencies can be easily and

TABLE IV
ESSENTIAL AMINO ACIDS IN SEAWEED MEALS

	<i>Cladophora rupestris</i> , Dunbar, Scotland				<i>Laminaria cloustoni</i> , Campbeltown, Scotland				Ascomael (<i>Ascophyllum nodosum</i>), commercial				<i>Rhodymenia palmata</i> , North Berwick, Scotland				Neptune's Bounty, commercial blend			
	In meal (%)		In protein ^a (%)		In meal (%)		In protein ^a (%)		In meal (%)		In protein ^a (%)		In meal (%)		In protein ^a (%)		In meal (%)		In protein ^a (%)	
	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d
Moisture	5.2				7.2				12.1				3.6				14			
Protein ^b	29	27.2	100	100	11.9	10.9	100	100	6.1	5.8			22.5	20.7	100	100	6.1	6.0		
Arginine	1.6	1.4	6.0	5.3	0.3	0.3	2.4	2.3	0.2	0.2			1.0	1.1	4.8	5.2	0.1	0.2		
Histidine	0.3	0.3	1.1	1.1	0.1	0.1	1.0	1.3	0.1	0.06			0.3	0.3	1.3	1.6	0.05	0.05		
Isoleucine	0.9	0.9	3.3	3.5	0.2	0.3	2.2	2.6	0.2	0.3			0.8	0.9	4.0	4.2	0.2	0.2		
Leucine	1.3	1.4	5.5	5.2	0.4	0.4	3.7	3.6	0.3	0.4			1.1	1.1	5.5	5.4	0.2	0.3		
Lysine	1.6	1.6	5.6	5.7	0.4	0.4	3.0	3.6	0.2	0.3			1.5	1.5	6.9	7.2	0.1	0.2		
Methionine	0.5	0.4	1.7	1.6	0.2	0.2	1.4	1.4	0.1	0.1			0.5	0.4	2.3	1.8	0.1	0.08		
Phenylalanine	1.0	1.0	3.0	3.5	0.3	0.3	2.1	2.7	0.2	0.3			0.8	0.8	3.2	3.9	0.2	0.2		
Threonine	1.0	1.2	3.9	4.3	0.4	0.4	3.6	4.0	0.2	0.3			0.9	0.9	4.4	4.3	0.2	0.2		
Tryptophan	0.3	0.3	0.9	1.1	0.04	0.09	0.4	0.8	0.04	0.07			0.2	0.3	0.9	1.3	0.04	0.06		
Valine	1.3	1.2	5.4	4.5	0.5	0.5	4.9	4.3	0.3	0.3			1.1	1.2	6.4	6.0	0.2	0.2		

^a Calculated as grams of amino acid per 16 g. of sample nitrogen.

^b Crude protein (nitrogen $\times 6.25$).

^c Analyses by R. W. Carroll, Quaker Oats Co., Chicago, using method of L. M. Henderson and E. E. Snell, *J. Biol. Chem.* **172**, 15 (1948).

^d According to C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).

TABLE V
COMPOSITION OF SEAWEED MEALS FROM COMMON BRITISH SEAWEEDS

Component	<i>Cladophora rupestris</i> (Chlorophyceae), Dunbar, Scotland (January 1955)	<i>Rhodomenia palmata</i> (Rhodophyceae), North Berwick, Scotland (January 1955)	<i>Laminaria</i> meal (<i>Phaeophyceae</i>) <i>L. cloustoni</i> frond, Campbeltown, Scotland (November 1954)	<i>L. cloustoni</i> stipe (bulked sample Jan./Dec. Oban, Scotland)	Ascomeal (<i>Phaeophyceae</i>) <i>Ascomphyllum nodosum</i> , Loch Maddy, Scotland (December 1954)	Neptune's Bounty, commercial blend (January 1955)
% of dry matter						
Total ash	29.3	27.4	21.8	37.6	24.5	20.6
Crude proteins	30.5	23.4	12.6	8.4	6.7	6.8
Fats	0.5	0.3	0.4	0.3	2.6	2.1
Crude fiber	16.6	2.1	5.0	9.6	8.4	7.7
Sodium	2.5	2.1	2.9	1.4	2.9	
Potassium	3.3	7.9	5.3	8.2	2.3	
Calcium	1.5	0.7	1.0	1.8	2.2	
Magnesium	0.7	0.4	0.6	0.7	0.8	
Iodine	0.1	0.3	0.5	0.3	0.1	
Phosphorus	0.3	0.6	0.3	0.3	0.1	
Silica	7.1	2.2	0.6	0.5	5.0	
Chlorine	6.3	9.7	5.9	12.5	1.9	
Sulfate	4.6	1.1	3.1	2.5	6.9	
p.p.m. in dry matter						
Cobalt	16.2	2.6	0.4	0.5	1.4	
Nickel	20.0	16.4	1.3	2.9	3.4	
Molybdenum	2.4	0.8	0.3	0.3	1.3	
Iron	4400	1355	437	446	1132	
Lead	38	28	7.9	5.4	<4	
Tin	<5	<5	<5	<5	<5	
Zinc	92	200	170	59	110	
Vanadium	24	29	1.0	2.5	5.9	
Titanium	550	100	18	26	114	
Chromium	6.4	3.4	1.4	1.3	2.9	
Silver	<0.7	1.0	0.2	0.9	<0.3	
Copper	31	48	4.6	5.0		
Manganese	1260	110	<20	47	45	
Barium	48	21	28	43	27	
Strontium	112	90	650	2500	560	
Vitamin content, γ /g. dry weight						
Thiamine	1.9	1.5	— ^a	—	— ^a	— ^a
Riboflavin	5.9	5.3	2.4	—	7.5	2.4
Niacin	26.2	28.9	19.4	—	12.3	10.8

^a No measurable amount.

Note: Total ash, crude proteins, fats, crude fiber and silica were determined by methods given in "The Fertilizer and Feeding Stuff Act 1926," H.M.S.O. Belfast, 1932; sodium potassium, calcium, magnesium, phosphorus, chloride and sulphate by methods given in C. S. Piper, "Soil and Plant Analysis," Interscience, New York, 1953; iodine by method of H. Baggesgaard-Rasmussen and G. Bjerresø, *Chem. Zentr.* 2, 2894 (1941); the trace elements cobalt to strontium by Dr. R. L. Mitchell, Macaulay Institute for Soil Research, Aberdeen, Scotland, using methods already reported (W. A. P. Black and R. L. Mitchell, *J. Marine Biol. Assoc. (United Kingdom)* 30, 575 (1952)); and the vitamins by Dr. R. W. Carroll, Quaker Oats Company, Chicago, using methods given in "Methods of Vitamin Assay," 2nd ed., Interscience, New York, 1951.

cheaply adjusted by the addition of bone flour or dicalcium phosphate. Seaweeds are a valuable source of iodine, partly present in the organic form as iodoamino acids which have been recommended for increasing the milk and butterfat production of dairy cows, for egg production, for fattening swine, and for reviving spermatogenesis in bulls and rams.

As a source of vitamins, seaweeds are unique, for not only do they contain the vitamins common to land plants, but also vitamins like B₁₂ which no doubt owe their origin to attached bacteria. Vitamin A is absent (63), but the brown seaweeds contain its precursor, β -carotene, as well as the brown pigment fucoxanthin, which may also be a precursor of this vitamin. In the B group they contain the vitamins B₁ (64), B₂ (65), and B₁₂ (66) in varying amounts, and it is noteworthy that several of the green seaweeds contain 0.5 to 1.0 γ of B₁₂ per gram dry weight, which is as high as that found in liver—one of the best known sources of this vitamin. Vitamin C (ascorbic acid) is as high in seaweeds as in lucerne (up to 8000 p.p.m.) (67). Recent work has shown that this vitamin can be stabilized by the addition of sodium alginate, which is one of the constituents of the brown algae (68). Doubt still exists as to the occurrence of vitamin D, but numerous workers have shown that seaweeds do have a distinct antirachitic effect (69, 70).

Recent work has shown tocopherol (vitamin E) to be present in the brown seaweeds in amounts varying from 1 to 35 mg. per 100 g. dry matter, being highest in the most exposed weeds (71). The presence of vitamin K (72), pantothenic acid, folic and folinic acids, and other growth-promoting factors (73) has also been noted. Recent work also indicates the presence of antibiotic substances (74).

Very little work has been carried out on the treatment of seaweed meals to improve their nutritive value. As far as is known, only one firm makes an attempt to reduce the mineral content and odor by washing and caramelization. Hydrolysis has also been tried, but the resultant products have not justified the treatment (75). There is an urgent need, therefore, for a cheap process to improve palatability, reduce the ash, and convert the alginate to an easily digestible form.

Work on the expression of cell sap of fresh algae has not produced any concentration of the proteins or likely improvement in nutritive value. Ensiling the fresh seaweed (51) before drying might improve its palatability and food value, but no feeding trials have been conducted with this product. In some countries the protein content of proprietary seaweed meals is increased by the addition of fish solubles to give a protein content of about 40%, and blends of this type are worthy of further consideration.

No work has, as yet, been carried out on the effect of the temperature of drying on the nutritive value of seaweeds.

e. Digestibility Trials

Seaweed consumption by the inhabitants of Western countries is very small, and for the most part it is eaten in times of distress or by small sections of the population who have acquired a taste for it. In certain Asiatic countries, however, the position is quite different and seaweeds in various forms are part of the normal diet of the people; but very few digestibility trials have been conducted.

The earliest recorded scientific trials were carried out in France (76, 77) when, during the 1914–18 war, the possibility of utilizing seaweed as a supplementary food for poultry, pigs, and horses was investigated. The animals accepted, digested, and assimilated the seaweeds, but it was noted that the seaweed appeared to remain completely undigested for the first few days of the trial and only after the sixth day, when no seaweed as such appeared in the feces, was digestibility excellent. Trials in Norway emphasized the differences which can arise in the nutritive value of seaweed meals, depending on the time of year when harvested. Lunde (78) conducted feeding trials with rats, pigs, horses, and poultry and showed that the addition of 5 to 10% seaweed was often beneficial. Ringen (79) carried out similar trials with pigs and sheep, tested two proprietary meals—one made from sublittoral weed (*Algit*) and the other from littoral weed (*Neptun*)—and obtained low digestibility values, particularly for pigs. The sheep utilized the meals better, with the sublittoral weed being superior to the littoral weed. Similar trials were carried out in Eire (75, 80), where digestibility trials with pigs indicated that *Laminaria* meal had a food value about two and one-half times that of potatoes and intermediate between hay and oats. Experiments with sheep were also conducted at the Albert Agricultural College in Eire. The most satisfactory meal (*Laminaria*) was that collected in the autumn, but it was only comparable in feeding value to meadow hay.

Trials in Germany, using a proprietary seaweed preparation (65% *Ascophyllum* meal), showed that it was uneconomical to feed this to fattening pigs (81), and when fed to chickens it had a detrimental effect at the levels fed (82), but, as no pure seaweed meal was available, direct conclusions as to its value were impossible.

In Canada, large-scale experiments with laying hens and chicks (83) showed that seaweed meal fed at the rate of 2.5 and 10% of the ration had little effect on the performance of laying hens from the point of view of mortality, egg production, eggshell strength, hatchability, and body weights. When chickens were fed on a ration containing *Ascophyllum* meal, substituted for ground oats at the 2.5, 5, and 10% levels, the differences in the mean final body weights were not significant.

The influence of the iodine in seaweed meal on the iodine content of hens' eggs (84) and on the physiological behavior of the laying hen has also been studied (85).

In Canada, pigs of comparable breeding have been fed barley and tankage or barley and fish meal with up to 6% of the ration in the form of *Asco-*

phyllum meal (86). No significant effect was observed with the seaweed meal. The rate of gain was favorable, and the carcass quality of the seaweed-fed pigs was similar to that of the controls.

In Great Britain, trials have been carried out with rats, dairy cows, sheep, pigs, and poultry (87). When the four main constituents of the brown algae were fed to rats, laminarin and sodium alginate at the 8 and 16% levels had the energy value of starch, and mannitol and fucoidin had a laxative effect. Rats were also used to assess the biological value of the algal proteins. The rats would not accept the dried, milled brown seaweeds, but *Laminaria cloustoni* frond, when mixed with casein, gave a net protein utilization of 30 (30). Experiments with cows to ascertain if the constituents of seaweed were readily utilized by the rumen bacteria and might, therefore, make possible the utilization of any non-protein constituents in the ration showed that only laminarin, alone or present in the weed, was utilized as readily as starch (88). Two seaweed meals (*Ascophyllum nodosum* and *Laminaria cloustoni*), when compared with oat feed as constituents of the concentrate ration for dairy cows, produced no significant differences in the mean milk yield and percentage of fat when fed at the 10% level (89). Trials on various farms, however, showed that the addition of $\frac{1}{2}$ pound per day of seaweed meal to the cow's ration significantly increased the butter fat content (90).

Sheep were used to determine the digestibility coefficient of four seaweed meals having wide variations in chemical composition. When fed at the high level of 20 to 24% of the basal diet, the *Ascophyllum* meal was poorly digested and even had a detrimental effect on the protein in the basal ration, but the *Laminaria* meal (January sample) gave a good digestibility for its crude protein (54.1%) and nitrogen-free extractives (71.8%) (87). Digestibility trials with the same seaweed meals used in the sheep trials were also carried out with pigs. In general, the results show that, as with sheep, the digestibility of *Ascophyllum* meal is considerably lower than that of the *Laminaria* meal. The *Laminaria* meal, however, gave a good digestibility for nitrogen-free extractives (88.5%), but a low digestibility for crude protein (7.1%). Group comparison feeding trials with pigs have shown that *Ascophyllum* meal can be introduced into the diet without affecting the growth rate or quality of the bacon (87). The meal was used to replace 5% of barley meal on a pound-for-pound basis when the pigs weighed 50 pounds, was increased to 12% at 90 pounds, and fed at this level to bacon weight. Experiments with one-year-old laying hens (91) have shown that 10% of the basal ration can be replaced by 10% of a seaweed meal with no ill effect on the birds, the egg production was maintained, and the birds remained perfectly healthy. In experiments with chicks, 5% of *Ascophyllum* meal provided all their vitamin A requirements and had a definite antirachitic effect (87).

V. UTILIZATION AND PROSPECTS

No accurate figures are available for the amount of algae harvested per annum for use in foodstuffs, but about 750,000 tons are collected annually from the shores and inshore waters of fifteen countries and processed by the chemical, food, and fertilizer industries. An indication of the magnitude of the industry can also be gained from the fact that, in 1951, France converted about 100,000 tons of littoral brown

seaweed into animal feed; Norway produces 10,000 to 12,000 tons, and California 18,000 tons of dry seaweed per annum. In Japan, the average production of dried indigenous seaweed for human consumption during the period 1940-49 exceeded 30,000 tons; from 1951 to 1953, 887,000 tons of fresh seaweed were collected. In 1953 alone, 304,000 tons (fresh weight) of seaweed were harvested; these included 47,000 tons of edible seaweeds (92).

Plankton harvesting is considered uneconomical in the Western Hemisphere but in the Far East, where labour is cheap and food scarce, increasing amounts are being recovered from the sea and used in foodstuffs. Thailand and Malaya,* for example, using "pong pang" bag nets, harvested 5000 and 2000 tons, respectively, in 1953 for use in shrimp pastes, but the organisms were largely zooplankton.

Although considerable work has been carried out on the mass culture of unicellular algae and the feasibility proved on the pilot scale, innumerable technical and engineering problems have yet to be overcome before a large-scale unit is erected. In countries like Israel and Japan, workers are confident that it will one day become economically feasible to mass-produce *Chlorella* as a protein source at a competitive price. The potentialities inherent in this method are enormous, and it seems likely that algal culture will one day occupy an important place in world food production. (See also Chapter 9.)

The green and red seaweeds containing 20 to 40% of crude proteins of high biological value could be utilized to a greater extent than at present, and surveys should be carried out and a cheap mechanical method of harvesting evolved. It would appear that the brown seaweeds, by virtue of their size and universal distribution, constitute the bulk of the world's seaweed resources. With crude protein contents, however, of 5 to 15% (dry basis), they cannot be regarded as sources of proteins, particularly in view of the fact that their unique composition limits the amount that can be fed without upsetting the metabolism of the animal. As an additive or supplementary foodstuff, trials have shown them to have a beneficial effect on health and digestion in general, contributing bulk, major elements, trace elements, vitamins, and growth-promoting factors.

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CHAPTER 31

INEDIBLE OILSEED MEALS

DON S. BOLLEY AND RAIFORD L. HOLMES

I. GENERAL INTRODUCTION

This chapter may seem at variance with the objective of this book; however, the composition and potential possibilities of the inedible meals justify their discussion. Although today they are classified as inedible, they may well serve in the future as a source of feed supplement and industrial protein. The protein contained in the inedible meals is quite similar to that of the edible meals. It becomes then a matter of removing or rendering harmless the toxic constituents. For example, when the inedible meals are hydrolyzed to amino acids, the toxic constituents are largely destroyed. The amino acid composition of the hydrolyzate is similar to that of other proteins.

The principal inedible meals are castor and tung. These are discussed at length. Meals from other oilseeds are mentioned only briefly.

II. CASTOR MEAL

1. Introduction

The castor plant has been known since ancient times. It occurs widely in the tropical and near-tropical regions as a perennial and is cultivated in the temperate zones as an annual. The castor bean (castor seed) contains approximately 50% oil and 18% protein. It is processed principally to obtain the oil; the meal (50% of the seed), which contains the protein (36%), is considered a by-product. For every pound of castor oil produced, there is an equal quantity of castor meal available.

The oil from the castor bean is a glyceride, but its composition is unique in comparison to the other common vegetable oils because the fatty acid of the glyceride consists of about 90% ricinoleic acid, which has the chemical formula $\text{CH}_3(\text{CH}_2)_5\text{CHOHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$. The hydroxyl group on the twelfth carbon of this acid is of particular interest, since this does not occur to any appreciable extent in any of the other common oils; its presence accounts for the unique properties and reactions of castor oil.

Castor oil is probably best known for its medicinal properties. Actually, the industrial applications for castor oil have increased to such an extent that its role as a laxative, although still important, is relatively minor. In the United States, it is estimated that less than 1% is employed medicinally. The largest use, about 60% in the United States, is in protective coatings. One of the largest uses in this field is as dehydrated castor oil. For this application, castor oil, a non-drying oil containing a hydroxyl group, is converted to a drying oil by elimination of the hydroxyl group. Other applications for the oil or its derivatives in protective coatings are as a plasticizer in nitrocellulose and as the fatty acid component in alkyds and epoxy resins. It is sulfonated and used in the textile industry as Turkey Red Oil. A number of castor products are employed in plastics and rubber. Castor oil once served widely as an engine lubricant. At present, sebacic acid from castor oil is used to make dioctyl sebacate, the only lubricant offering satisfactory performance over the tremendous temperature ranges encountered in jet engines. Castor oil is employed in hair tonics, salves, and lipsticks. Also produced are numerous derivatives of the oil, which find a large variety of applications.

2. Production and Trade

a. General Information

About 500,000 short tons of castor beans (Table I) are gathered and processed annually, producing approximately 250,000 tons of meal (1, 2). This quantity has been fairly constant over the past twenty years (3). Well over one-half of the world production of castor beans originates in Brazil and India (4). Both of these countries were major exporters of beans, but India has practically ceased export, and Brazil has reduced its exports drastically. Neither country exports any appreciable quantity of meal.

b. United States

Exports of castor beans from Brazil to the United States in 1955 were estimated at 31,695 tons but six years previously Brazil shipped over 100,000 tons annually to this country. Beans are also imported to the United States from Ecuador, Haiti, British East Africa, The Union of South Africa, and Angola. Total bean imports to the United States have declined from the record high of 197,225 tons in 1941 to 37,585 tons in 1955. The trend of increasing oil exports and decreasing seed exports results in less meal being available in the United States, which is the largest consumer of the oil. It is estimated that 70,000 tons of meal were produced in 1947, 45,000 tons in 1952, and 25,000 tons in 1955.

Originally, castor beans were widely grown in the United States. One hundred years ago there were a number of crushing plants producing castor meal and oil from domestic seed. At the beginning of the twentieth century pro-

TABLE I
CASTOR BEAN PRODUCTION^a

Country and continent	Production			
	1945-49 average	1954	1955	1956
		short tons		
North America	6,340	12,075	6,670	12,870
Mexico	3,195	3,300	2,200	6,600
United States ^b	—	5,400	—	2,500
Europe (exclusive of U.S.S.R.)	6,625	5,770	4,665	3,770
Asia (exclusive of U.S.S.R.)	176,135	176,825	198,220	204,280
Iran	6,500	8,270	8,800	8,800
India	133,728	115,360	138,880	141,120
Thailand	550	15,881	14,440	18,000
South America	219,690	208,100	189,580	182,130
Brazil	207,180	187,260	175,700	168,200
Ecuador	2,800	10,643	7,151	7,000
Africa	28,100	54,140	43,140	40,340
Tanganyika	1,230	10,762	8,814	6,500
Uganda	—	8,536	3,749	—
Angola	5,063	3,130	1,448	—
Union of South Africa	—	4,230	—	—
World total (estimated)	492,000	536,910	522,275	523,390

^a According to *U. S. Dept. Agr. Foreign Agr. Service Circ. FFO 2-57* (March 26, 1957).

^b Production in 1953 was 25,675 tons.

duction began to decrease, and shortly after World War I the domestic growth of castor beans disappeared. Increasing quantities of beans were imported and crushed in the United States, however, resulting in the production of an increasing amount of meal. This has reached a maximum and now again is declining.

The desirability of a domestic crop has been recognized for some time. Before domestic production of castor beans could be economically feasible, the yields per acre had to be increased, the shattering of the mature capsules had to be overcome, and the size of the plants reduced so that machine harvesting could be used. An intensive agronomic research and plant breeding program conducted by scientists in Government and private agencies resulted in the development of new varieties of castor beans in which these disadvantages were largely eliminated. There are now numerous dwarf, non-shattering varieties available that produce a high yield of seed per acre. Hybrids have been developed that are earlier in maturing and give considerably higher yield than the inbred types. Yields of over 5000 pounds per acre have been reported in experimental plantings (5), and 4000 pounds per acre have been frequently obtained on irrigated land. This would be equivalent to 2000 pounds of oil and 2000 pounds of meal or 720 pounds of protein per acre. The average present

yield of beans on irrigated land is probably about one half this quantity, and for dry land about one-sixth.

In 1950, a Massey-Harris harvester was put in commercial use, and more recently, the Boardman machine.* Special dehullers and conveyors were also developed. In 1951, about 10 million pounds of oil and meal were obtained from domestic beans. This increased to 25 million pounds in 1953, but declined sharply in 1954, owing to reduction of the support price for the bean.

Castor grows well in Oklahoma, Texas, New Mexico, Arizona, and south California. It is often grown as an ornamental plant and for this purpose will thrive in almost all parts of this country. Excellent summaries on domestic development of the plant have been published (6, 7).

Castor beans are crushed and oil and meal produced in the United States in several plants located in the Northeast and on the Pacific Coast in California. A small amount of domestic crushing is carried out by cottonseed mills in the Southern states. The meal usually is marketed as castor pomace. Its price at the shipping point since 1945 has ranged from \$20 to \$40 per ton, the higher price is now prevalent because of short supply. In 1953, seed costing \$0.10 per pound yielded \$0.109 worth of oil and \$0.007 worth of meal. In 1954, the price of seed lowered to \$0.06, yielding approximately \$0.079 worth of oil and \$0.007 worth of meal.

c. Brazil

The principal production and processing areas in Brazil are located in the states of Bahia, São Paulo, Pernambuco, and Ceará; approximately 60,000 tons were grown in Bahia in 1955. The yield per acre ranges from 740 to 14000 pounds, with the highest yield in Bahia. Most harvesting is by hand, although some dwarf and non-shattering varieties have been developed and are being tested, foreshadowing mechanical harvesting. In 1956, beans sold for \$150 per ton, oil for 16 cents per pound, and meal for \$70 per ton, the oil representing over 80% of the value of the products of the castor bean. The amount of beans grown is increasing somewhat, and the portion being processed domestically is increasing continuously. Conventional types of oilseed processing equipment are being used.†

d. India

Of the 1,273,000 acres devoted to castor production in India in 1954-55, half were in the state of Hyderabad. About one-half of the castor seed produced in India (about 66,000 tons in 1954-55) was

* Developed by the Boardman Company, Oklahoma City, Oklahoma.

† R. C. Antonissen, private communication.

crushed in power-driven mills, yielding 30,000 tons of oil and 35,000 tons of meal. Most of the castor seed was crushed in the State of Bombay. In March, 1955, the price of castor beans in Bombay was equivalent to about 2.6, 6.8, and 0.95 cents per pound for seed, oil, and meal, respectively (8). As in other countries, there are many varieties of castor grown, and a continuous effort is being made toward improvement. The beans are gathered by hand. Production has been fairly stationary for the last four to five years, but processing is likely to increase on account of increasing industrial expansion.

3. Appearance and Growth Characteristics

Castor seeds are commonly referred to as castor beans. This is not botanically correct, since the plant is not a legume but belongs to the Spurge family. The terms castor bean and castor seed are used interchangeably.

The castor plant, *Ricinus communis*, probably originated in India. It was given the generic name *Ricinus*, Latin for tick, because of the resemblance of the bean to the appearance of a tick. In temperate zones the plant is grown as an annual, but in the tropics it is perennial and becomes a small tree which sometimes reaches a height of 30 feet. It is tolerant of a wide range of climatic conditions but needs a moderate amount of rainfall. In order to produce a satisfactory quantity of seeds, it must have a relatively long growing season of at least 190 days.

Three seeds (castor beans) are encased in a spiny (spineless varieties are known) outer shell which is referred to as the hull (Fig. 1). The hull and the three seeds are the fruit of the castor plant which grows in clusters or spikes. In hand harvesting, the clusters are cut or pulled from the plant and allowed to dry. When harvested mechanically, the fruit is usually dry and pulled off by machine. The seeds are freed from the outer shell or hull by drying or with dehulling machinery. The hulls have some fertilizer value and are often spread back on the ground. Harvesters have now been developed that "pick" the seed and dehull in one operation.

Castor seeds vary considerably in size and appearance. They are usually mottled light brown, although they range from nearly white to all black. Some seed coats are red, but the beans grown in the United States are usually mottled gray. Some varieties weigh less than 0.1 g. per seed, whereas others, particularly those growing wild in the tropics, may weigh over 1 g. Cultivated seeds in the United States weigh 0.2 to 0.3 g. apiece. The seed contains approximately one-half oil, one-fourth coat, and one-fourth flour. Immature seed is high in moisture content,

which is reduced to about 5% on maturity. There is little change in oil or protein content on prolonged dry storage.

There are two easily separable parts, the seed coat and the kernel. The kernel, hereafter referred to as meats, contains the oil and most of

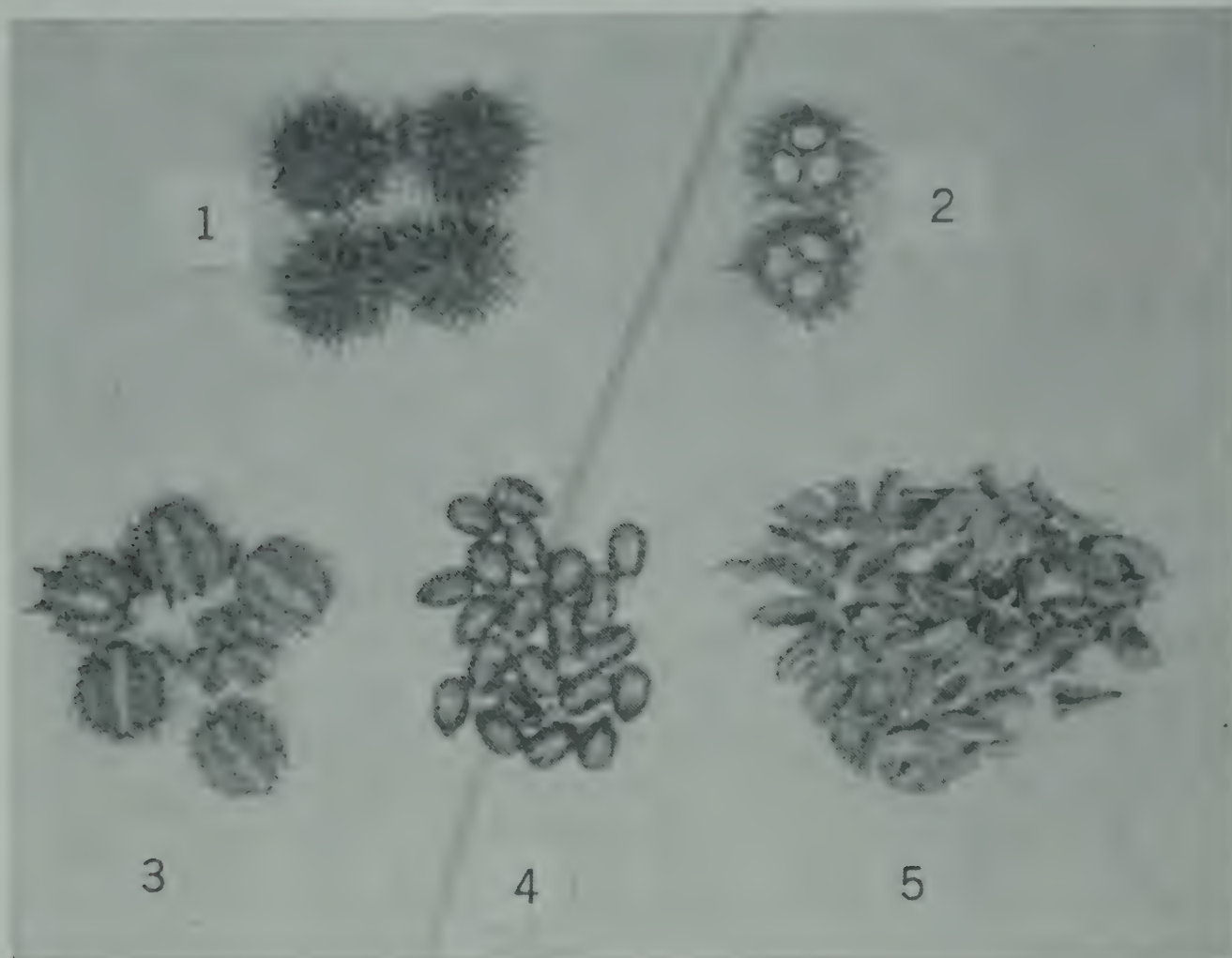


FIG. 1. Castor fruit (capsules), seed, and hull: (1) mature castor fruit, sometimes called capsules; (2) section through fruit; (3) dried fruit; (4) seeds; (5) hulls.

the protein. Most of the weight of the kernel consists of endosperm which encloses a flat embryo. When the oil is removed from the kernel, the residue is known as high protein meal or flour.

4. Processing

Oil is removed from the castor beans by the same general procedures as for other oilseeds. These consist of the use of a cage hydraulic press and more recently the mechanical screw press. With the hydraulic press, the whole, cleaned beans are heated and pressed at 60° to 70°. This produces the No. 1 castor oil. (For pharmaceutical grades in the United Kingdom, the maximum permitted temperature is 40°.) The press cake, frequently called "shells," which contains about 15% oil, is extracted by contact with heptane or hexane solvent for several hours at about 90° to reduce the oil content to about 1%. Oil so produced is known as No. 3 castor oil. After the miscella is filtered off, the residual solvent in the cake is removed by steaming, at a maximum temperature of 120°. These temperatures to which the material is heated and the

presence of moisture are important for detoxification of the meal. Castor meal produced by hydraulic pressing followed by solvent extraction is known as castor pomace.

There are mills throughout Mexico and Central and South America that are presently processing castor beans in mechanical screw presses (9). For the double pressing (two successive pressings), the beans are warmed to 150°F. and prepressed in the mechanical screw press. The prepress cake contains approximately 20% oil. It is dried to a moisture content of 4 to 5% by heating to 200°F. and is then pressed again to furnish the No. 3 quality oil. The resulting press cake then contains 5 to 7% oil and has a moisture content of about 5%. Some plants screw-press castor beans in a single operation after drying to a moisture content of 5 to 6%. Slightly more oil, 6 to 8%, and a moisture content of about 8% is usually left in these cakes. Some work is being done with decorticated beans, i.e., castor meats. This results in a meal with considerably higher protein content. Castor meal and castor flour (extracted meats) have been produced by solvent extraction. The process is not used extensively, however.

The castor pomace or meal is ground and usually stored in paper bags. It may be kept for an indefinite length of time when stored properly in a dry place.

5. Composition

a. General Information

Castor beans contain toxic components. Since these are not extracted with the oil, they are a component of the meal, unless destroyed during processing. The principal toxic component is a protein, ricin. There is also present a powerful allergen that is more difficult to inactivate than the ricin. A unique alkaloid, ricinine, which is only slightly toxic has also been isolated from the meal. Ricin, the allergen, and ricinine have been studied extensively and will be discussed separately. They are contained in the flour (oil-free endosperm) which forms about 50% of the meal. The other half of the meal is the seed coat. Very little is known concerning the composition of the seed coat except that it is high in cellulose content, contains some pentosans and pigments, and has a low protein content. The carbohydrates present in the meal and flour have attracted practically no experimental interest to date.

Table II gives the gross chemical analysis of castor pomace. Spectroscopic analysis (10, 11)* shows the presence of small quantities

* Baker Castor Oil Company, private communication.

TABLE II
GROSS CHEMICAL COMPOSITION OF CASTOR POMACE^a

Constituent	Content
	%
Fat	1.75
Fiber	13.29
Nitrogen (as NH ₃)	6.58
Crude protein	33.8 (calc.) ^b
Phosphorus (as P ₂ O ₅)	1.96
Calcium (as CaO)	0.10
Magnesium (as MgO)	0.23
Potash (as K ₂ O)	1.25

^a According to W. B. Hawke, *Am. Fertilizer* **78** (2), 9 (1933).

^b Nitrogen \times 6.25.

of numerous elements, including the "trace elements" necessary for plant growth. The crude protein content of the meal or pomace calculated from the nitrogen content ($N \times 6.25$) is about 36%; in the flour it is 66 to 70%. It is estimated that the protein consists of 60% globulins (potential industrial protein), 16% albumins (contains the ricin), 4% proteoses (contains the allergen), and 20% glutelins, conjugated proteins, and non-protein nitrogen compounds.

Amino acid analyses of castor pomace and castor flour are given in Table III. The amino acid composition of castor pomace is similar in general to that for other oilseed proteins (12).

b. Ricin

Ricin is in the albumin, the water-soluble fraction of the undenatured protein. Considerable interest has centered on the extreme toxicity of this toxalbumin, and excellent reviews have been prepared by Van Heyningen (13) and Jones (14). (In some reviews ricin is classified as a globulin (13).)

Dixon in 1887 (15) was apparently the first to recognize and isolate the toxic protein from the castor bean. It was named ricin by Stillmark in 1889 (16). Ehrlich (17) and later Cushny (18) observed that the toxicity of ricin is destroyed when a water solution of the albumin is boiled. Müller (19), Jacoby (20), Reid (21), and Brieger (22) attempted to destroy the toxicity of ricin by treatment with proteolytic enzymes. Since they were not successful, their experiments cast doubt on the protein nature of ricin; however, Kobert (23) showed that, given enough time, the toxicity diminishes with the extent of the digestion. Ramon (24) recognized ricin to be similar to other toxins

TABLE III
AMINO ACIDS IN CRUDE CASTOR PROTEIN

Amino acid	Pomace			Flour	
	grams of amino acid per 16 g. of sample nitrogen				
Arginine	11.0 ^a	10.0 ^b	9.8 ^c	12.9 ^b	12.2 ^c
Aspartic acid	4.6	—	—	—	—
Glutamic acid	18.0	—	—	—	—
Histidine	2.5	1.7	1.7	2.1	2.0
Isoleucine	5.3	4.6	5.2	5.3	5.7
Leucine	7.2	5.6	6.3	6.4	6.3
Lysine	3.1	3.0	2.8	3.4	3.3
Methionine	1.5	1.5	1.5	1.8	1.9
Phenylalanine	4.2	4.7	3.2	5.2	3.5
Proline	3.9	—	—	—	—
Threonine	3.6	3.2	3.3	3.8	3.6
Tryptophan	0.8	1.1	0.8	1.4	1.1
Tyrosine	3.2	—	—	—	—
Valine	6.6	5.4	6.8	6.7	6.9
Crude protein, %		39.8		65.03	

^a All figures in this column according to R. Kodras, C. K. Whitehair, and R. MacVicar, *J. Am. Oil Chemists' Soc.* **26**, 641 (1949).

^b All figures in this column according to C. M. Lyman, K. A. Kuiken and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).

^c All figures in this column according to R. W. Carroll, Quaker Oats Co., private communication.

such as diptheria and cobra venom, both of which can be rendered non-toxic by treatment with formalin without losing their antigenic properties. The immunizing power of such preparations can be determined *in vitro* by the method of flocculation. Heller (25) observed that dry heat does not destroy the toxicity of the protein. The influence of temperature and pH on the rate of denaturation of ricin has been studied (26).

In 1905, Osborne and his collaborators (27) made an extensive study of the isolation of ricin. About 5% of crude ricin (50% purity) was isolated from castor flour. It was extremely toxic, injection of 0.001 mg. per kilogram of rabbit causing death (see also Van Heyningen (13)). The globulin isolated at the same time was not particularly toxic; its slight toxicity was probably due to some ricin impurity present. The proteose fractions also were not toxic. Funck (28) reported that the toxic constituents comprise 2.8 to 3% of the whole seed (about 10% of the castor flour). Kabat *et al.* (29) prepared a purified ricin that had an isoelectric point of about 5.3, a molecular weight of 80,000, and a partial specific volume of 0.75. Kunitz and McDonald (30) isolated and crystallized ricin by means of a sodium sulfate fractionation technique. Moulé (31) isolated pure ricin by column chromatography and made amino acid determinations. She also determined its physicochemical properties (32). Her product showed two components by electrophoresis but had the same toxicity

as the crystalline sample of ricin prepared by Kunitz and McDonald. Karrer *et al.* (33) studied the amino acid composition of ricin. It was typical of a vegetable protein and gave no clue as to its powerful toxic action.

The physiological action of ricin indicates it to be a typical toxalbumin similar to the bacterial toxins. It is apparently toxic to all species of mammalian animals, since it affects man, sheep, horses, cattle, pigs, dogs, rats, and guinea pigs. Ducks and chickens are also affected but are somewhat more tolerant. Its oral toxicity is probably related to its resistance to proteolysis. The toxic response has been described by Jones (14), Van Heyningen (13), and Corwin (34). If a lethal dose is taken, there is first a latent period, followed by vomiting, diarrhea, and cramps. The termination may be coma, convulsions, or collapse of the blood circulation. In animals, there is much inflammation of the stomach accompanied by edema and hemorrhage. Ricin also affects the eyes, causing intense inflammation.

Like the other toxalbumins, ricin can agglutinate red blood corpuscles. This offers a convenient test for its presence; the macroscopic tube agglutination test (35) has been used.* Another typical characteristic is the action of ricin as an antigen. If increasing quantities of this toxin are carefully given, it is possible to render an animal immune to a dose more than a thousand times as great as that which would have killed without immunization. Formaldehyde-detoxified ricin can also be used to produce immunization.

c. Allergen

The allergen in the castor bean or meal is considered to be a proteose. Similar allergenic proteoses have been observed in a number of other seeds including cottonseed, flaxseed, almond nuts, Brazil nuts, and mustard seed (36). Proteoses are soluble in water, non heat-coagulable, but may be precipitated by saturated ammonium sulfate.

Alilaire (37) was apparently the first to describe human hypersensitivity to the castor bean. He believed the allergen to be identical with ricin. Ratner and Gruehl (38) performed a comprehensive series of experiments with castor bean dust on guinea pigs which demonstrated the difference between ricin and allergen. Immunity to ricin can be brought about by repeated inhalations of small amounts of castor bean dust; sensitization to the allergen is caused by the same means. Barnard (39) stated that the allergen was water-soluble, alcohol-precipitable, heat-stable, and nondialyzable. Grabar and Koutseff (40) prepared the pure allergen by various steps including ultrafiltration and dialysis. They obtained 30 g. of substance from 500 g. of whole seed which, when tested on an allergic human subject, shows a positive skin reaction at a

* Baker Castor Oil Company, private laboratory report.

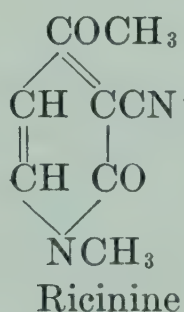
dosage of 0.01 mg. The extract was free from all toxicity because when injected into a mouse, at a dosage of 1.5 g. per kilogram of animal weight, it was not fatal.

Figley and Elrod (41) showed that the unusually high incidence of asthma in a Toledo school and in the surrounding neighborhood was caused by castor pomace dust expelled from ventilating stacks of a nearby linseed and castor bean mill. Vaughan (42) found that castor bean allergen was the causative factor for several asthmatics who were exposed to a fertilizer containing castor bean pomace. Bernton (43) has reported a case of allergic attacks in a man which incapacitated him for two weeks at a time. The attacks followed contact with an apparently clean burlap sack which at one time undoubtedly had contained or had been in contact with castor bean pomace.

Spies and his associates (44-49) have reported on their comprehensive study of allergens which include those from the castor seed. They obtained a yield of 1.8% of a non-toxic allergen from castor bean, 10^{-10} g. being sufficient to cause a welt on a susceptible human subject. The allergen prepared according to Spies was found to have a nitrogen content of 18.3%, sulfur 2.3%, and carbohydrate 3.12%. Its amino acid composition indicated a very high content of arginine (26%) compared to that normally in crude castor protein (12%). The preparation was found to be about 50% dialyzable, the dialyzate having about the same allergenic potency as the original. Its chemical properties were similar to those of the allergen isolated from cottonseed.

d. Ricinine

An alkaloid, ricinine, has been isolated from the castor oil seed by a number of investigators. This alkaloid, which should not be confused with the protein, ricin, has the following formula:



A good review of its proof of structure is given by Henry (50). Tuson (51) was apparently the first to isolate ricinine from the castor seed. He obtained the alkaloid by boiling with water, filtering, evaporating the filtrate to a thick syrup, and extracting with boiling alcohol. Ricinine was found to be soluble in water and alcohol, and very slightly soluble in ether and benzene. Schulze (52, 53) obtained ricinine by extracting the germinated seed with alcohol and then treating the aqueous solution with tannic acid and lead acetate. After purification with charcoal and crystallization from absolute alcohol, the product was found to have a melting point of 193° .

The absolute amount of this alkaloid constantly increases with plant growth (54). Although ricinine is not markedly toxic to human beings (50), it shows toxicity comparable with that of lead arsenate against codling moth larvae (55, 56).

It has no effect on grasshoppers (57). Evans (58) studied the chemical properties of ricinine using the methods of isolation described by the previous authors.

e. Phytin

Castor meal and pomace have a relatively high phosphorus content. Koehler (59) reported 4.82% phosphorus (as P_2O_5) in the castor beans. Phytin phosphorus represents 91% of this total. It has been observed (60) that castor seeds contain higher phytin content than most other common seeds. One writer found 5.9% phytin in castor flour.

f. Lipase

Castor flour (residue after oil is removed from decorticated castor beans) contains a powerful lipolytic enzyme. In fact, crude castor lipase is castor flour when prepared without heat treatment. The literature on the subject is extensive, and no attempt will be made to treat it exhaustively here.

Green (61) simultaneously with Sigmund (62) in 1890 was apparently the first to establish the presence of a fat-splitting enzyme in castor beans. Connstein (63) found the lipase to be insoluble in water. Its activity is reduced by contact with water but stabilized by fats. It is sensitive to ethanol but not affected by benzene, ether, or carbon disulfide. The lipase is rapidly inactivated by alkali and functions only in a neutral or slightly acidic medium. Lipase can act without the addition of any acid, although small amounts of acids accelerate the action (64). The greatest activity is with an acetic acid buffer, at pH of 5 (65). Nicloux (66-70) took out a series of French, German and English patents in 1903-04 on the use of the emulsion form of lipase. Falk and his collaborators (71-74) reported on a rather comprehensive series of experiments with castor bean lipase. Lipase (castor flour) was used in the hydrolysis of methyl acetate, olive oil, and ethyl butyrate. The optimum temperature for the action of ricinous lipase is 35° (75, 76). It can be heated as high as 165° in the presence of fat without inactivation, although it is rapidly inactivated even at 60° in the absence of fat. It functions more efficiently in a water-in-oil emulsion. Sachs (77) remarks that the advantage of enzyme-splitting to produce fatty acids is that light-colored acids are obtained, essentially unaltered in composition by the process. The disadvantages are the loss of fatty material occasioned by the formation of the middle layer, the long time required for the reaction, and the fact that the splitting is never complete. Haley and Lyman (78) published an excellent article on castor bean lipase, giving details of its method of preparation and use. Longenecker and Haley (79) have studied the rate of hydrolysis of various oils by ricinus lipase. It was

noted that a ten-year-old sample of lipase still had considerable activity. An analysis of the data on the basis of the number of moles hydrolyzed revealed that the lipase showed no specificity in its attack on glyceride molecules containing carbon chains of different lengths. A procedure for preparing an active lipase cream of reproducible activity from castor beans was described by Rose (80, 81).

6. Uses

a. Toxic Properties

It is apparent that the principal toxic properties of the castor bean are due to the ricin and allergen. A comprehensive article on the detoxification of castor seed pomace was published by Kodras *et al.* (82). It was stated that autoclaving for 15 minutes at 125° destroyed ricin. All properly prepared commercial castor pomace has been heat-treated and the ricin sufficiently detoxified as not to give a positive blood agglutination test. As solvent extraction becomes more widely used for extracting oil from the castor bean, however, continuous precaution should be observed that the meal has had sufficient heat treatment to detoxify the ricin.

The situation with allergen is less satisfactory. In the first place, smaller traces of allergen serve to affect a sensitive subject. Also, the allergen component is more difficult to detect. Undoubtedly, the detoxified pomace has a lesser allergic effect than castor flour that has not been heat-treated. It has frequently been observed that the subjects who are only slightly sensitive to castor allergen are not bothered by exposure to the pomace dust, but they are considerably affected by non-heat-treated castor flour.

There is also evidence for the presence of an unknown ulcerative factor. Carmichael (83–85) observed that ulcers are often formed at the site of the injection of ricin solutions that have been detoxified.

An attempt has been made to breed a ricin-free variety of castor bean (86); however, this was not successful. One author has shown that the variation in ricin content of the seed is due more to its age and storage conditions than to variety.

b. Fertilizer

Castor pomace or meal finds its principal application as a fertilizer. It owes such value to its high nitrogen (6.4%), phosphoric acid (2.55%), and potash (0.96%) content. It has added value since it contains many secondary plant nutrients and forms humus. Castor pomace fertilizer slowly decomposes in the soil, thus assuring a steady supply of nutrients; and its retention of moisture places it in the same order as

peat. It has particular value for tobacco, potatoes, lawns, and truck crops. In India the cake is considered a valuable fertilizer especially for sugar cane and paddy. Comparison with other organic fertilizers rated it high on the basis of nitrogen nitrified (87).

c. Industrial Protein

Industrial protein from oilseeds is usually prepared from the globulin fraction. (See also Chapter 10.) This is the principal protein present in castor meal, and it may be isolated by the conventional procedures of extraction with alkali and precipitation with acid. One author's experience has indicated that relatively poor yields and quality are obtained from commercial pomace which has been detoxified. Yields of apparently good-quality material (about 50%) are obtained from the solvent-extracted meats or flour. The protein so obtained appears similar to the globulin from other seed sources such as soybean and linseed. Ricin and allergen are not globulins and hence should not be contained in the highly purified, industrial protein preparation. Further study is necessary, however, to determine how effective the various purification procedures are in reducing these toxic constituents to a tolerable quantity.

Ritthausen, in a series of articles beginning 1879 (88-90), described a method for the preparation of a crystalline globulin from the castor bean. His method of crystallization was to extract the pomace with a 10% warm sodium chloride solution and then allow the solution to cool gradually. The proteins crystallized in the form of octahedra which were found to be soluble in glycerin. Osborne, beginning in 1892, published an extensive series of studies on proteins including those from castor bean (91-96). His work was thorough and may still be read with profit. Osborne's initial experiments consisted of the preparation of crystals which he analyzed and compared to the data of Ritthausen. He extracted the protein with salt solution and dialyzed the extract to permit crystallization. His amino acid composition indicated it to be similar to other seed proteins.

A 49.5% yield of crude industrial protein was obtained by extracting the meal at 40° to 45° for 2 hours with an alcoholic sodium hydroxide solution (97). The protein was recovered by precipitating with 3.5% hydrochloric acid. Investigators in Russia (98) treated the meal with a 10% sodium chloride solution and recovered the protein by acidifying with sulfuric acid. A yield of 22% was obtained. They found it high in content of glutamic acid and arginine. In India, Kamath and Kulkarni (99) prepared the protein by alkali extraction and coagulation with acetic acid or sulfur dioxide. Preliminary testing indicated it to have value for water paints, fibers, or glues. A Sherwin-Williams patent (100) discloses the recovery of proteinaceous material by alkali treatment and subsequent acidification. The resulting protein is stated to be detoxified. It has been reported (14) that textile fibers made experimentally from castor seed protein compare favorably with those made from the proteins of soybeans, peanuts, and casein.

d. Feed

Castor pomace has at various times been used in mixed feed for farm animals, and its amino acid composition would seem to make it suitable for this purpose. Because of the known toxicity of its components, however, this practice may be hazardous. Even if it were possible to remove completely the ricin, there would still be the possibility that the allergen component would sensitize the animal. Apparently, fowls are more resistant than mammals to the toxic principles (101). There is also the possibility that the residual castor oil in the meal might have harmful physiological effects if used in feed over extended periods. Since the meal is potentially a good source of feed, except for its toxic constituents, there does exist a possibility that future research will indicate how this material may be utilized. Reports have indicated that it may be used for feeding fish which are apparently not affected by the toxic properties.

e. Miscellaneous

Castor bean meal has been used in Manchuria as a source of glutamic acid in the preparation of sauces and condiments (12). Various claims are continuously cropping up for the use of the castor bean plant in combating pests. Most of them have proved to be unfounded. There have been numerous reports that moles may be eliminated by placing castor beans in their runs. Evidence as to their effectiveness seems to be contradictory. The moles' appetite for castor beans probably depends on the availability of other food. Castor meal has also been used in fire-fighting foams in a similar manner to that developed for soybean meal.

7. Trends

There has been a trend in the past decade for less beans to enter into world trade. Since the amount of oil consumed and produced in the various countries is about the same, this means that greater quantities of beans are being processed in the countries where they are being grown. In particular, Brazil has been sending increasing quantities of oil to the United States along with lesser quantities of beans. Thus, there is more meal produced in the bean-growing countries such as Brazil and India and less in the large castor oil consuming countries such as the United States.

An influence on this trend might be the recent domestic program for reintroducing the castor bean crop into the United States. At present it furnishes only a small fraction of the demand, although potentially there are sufficient areas available to supply easily all the necessary

castor beans. Economic factors will control further expansion of the domestic crop. The price to the farmer for the seed apparently has a control on the size of the crop. This in turn depends on the price of the oil from the seed and to a far lesser extent on the price of the castor pomace. If the castor pomace could attain a greater value, it would obviously do much to assist the United States domestic program as well as world production.

Greatly improved strains of seed for the growing of castor beans have been developed by plant breeding. Hybrid seeds are now available that may at least double the average yield. This should make the crop considerably more attractive to the farmer and result in greater acreage. Improved harvesters and handling equipment are also available.

There is apparently a recent trend to process the castor bean by the mechanical screw press. The castor pomace or meal obtained from this processing when used as fertilizer would probably be similar to castor pomace obtained by the conventional hydraulic pressing-solvent extraction. Whether it would serve as a raw material source for the better utilization of protein is yet to be seen.

Experiments are being conducted on decortication and processing the decorticated bean. The resulting meal will have a protein content of about 70% and should be much lighter in color than the present castor pomace. It offers some interesting possibilities for industrial and feed use, if success can be attained in eliminating the toxic and allergenic constituents.

III. TUNG MEAL

1. Introduction

Tung meal is a by-product of the production of tung oil or China wood oil from the fruit of the tung tree. The term refers to the ground mixture of tung kernels and shell from which the oil has been extracted either by pressing or by solvent extraction, whether it is in the form of a hard cake from the presses or a finely ground meal.

Tung oil is used in the manufacture of high-grade varnishes and protective coatings, especially in floor and deck varnishes where resistance to wear and water is needed. Because of its unique chemical composition, tung oil when blended with certain resins produces a tougher, more durable film than does any other vegetable drying oil. It is also used in wrinkle finishes. Its unique properties are due to its high content (about 80%) of eleostearic acid, $\text{CH}_3(\text{CH}_2)_3(\text{CH}=\text{CH})_3(\text{CH}_2)_7\text{CO}_2\text{H}$, an 18-carbon acid similar to linolenic acid in having three carbon-to-carbon double bonds, but unlike linolenic acid in that the double bonds are closer together (conjugated). Tung oil is the only commercial oil that contains this acid.

2. Production and Trade

a. Countries

(1) *China*. Tung oil has been produced in China from time immemorial and until the last decade China was the source of nearly all the world's supply. The first knowledge of tung oil in Europe was brought back in the thirteenth century by Marco Polo, who mentions it in his famous "Travels" (102), written after a sojourn of twenty-five years in China. Tung oil at that time was used in China for protective and decorative coatings and still finds its principal application in that field.

In China, tung trees are grown mostly on land unfit for food crops and receive little or no cultivation. It has been estimated (103) that before World War II China's production of tung oil averaged about 264 million pounds per year. The United States alone in 1937 imported 137 million pounds from China. The production of oil in China declined during and after World War II as a result of war and political conditions; in 1946 it was estimated to have decreased by one-fourth to one-half as compared to prewar production (103).

(2) *United States*. The tung tree was introduced into the United States in 1905 and specimens planted at many locations over the southern and western states. Only those trees in the southeastern part of the country thrived. The first commercial planting of tung trees in the United States was made in Florida in 1924 (104), and the first commercial production of oil (60,000 pounds) was at Gainesville, Florida, in 1932 (105). By 1954 the acreage in the United States had increased to about 200,000 and the production of oil to about 40 million pounds.

In the cultivated orchards of the United States, the trees are planted on the contour about 70 trees per acre and are cultivated and fertilized, cover crops usually being planted between rows until the trees become large enough to crowd out undergrowth. It is important that suitable land be selected for an orchard, as the soil has to be especially well drained. In good crop years in the United States the average yield of tung fruit has been 0.5 to 0.75 ton per acre, including trees of all ages on poor and good soils. Exceptional plantings have yielded 3.5 to 4.0 tons per acre from older trees. The yield from year to year is variable because of damage from late frosts, such a frost having destroyed practically the entire crop in 1955.

(3) *Argentina*. The first tung tree plantings in Argentina, from seed obtained in the United States, were made in 1928 in the Province of Misiones (105), where most of the tung plantations in Argentina are located. Argentine production of tung oil reached 41 million pounds in

1953, and in 1955 is expected to be about 25 million pounds (106). The average yield of nuts in Argentina is slightly less than 1.0 ton per acre.

(4) *Others.* Plantings of tung trees were also made in Brazil and Paraguay about 1928. Other countries also produce small amounts of tung oil, but most of it is produced in China, the United States, Argentina, Brazil, and Paraguay.

b. Tung Meal

Since most tung meal is used locally for fertilizer, there are few statistics on its actual production. The United States Bureau of the

TABLE IV
PRODUCTION OF TUNG MEAL IN VARIOUS COUNTRIES 1945-54^a

Production of meal					
Year	China	United States	Argentina	Brazil	Paraguay
	thousand of pounds				
1945	No statistics available but probably about 250,000,000 pounds per year.	14,226	5,198	277	164
1946		22,042	5,831	770	611
1947		20,429	3,736	540	188
1948		22,464	9,617	947	680
1949		32,128	15,964	1,942	3,134
1950		14,438	24,480	1,582	3,444
1951		17,796	34,800	2,196	4,764
1952		52,314	11,880	2,040	4,080
1953		46,168	40,800	2,400	9,240
1954		20,222	34,392	—	—

^a The production of meal for the United States for the period 1950-54 are from the reports of the U. S. Bureau of the Census. All other figures are calculated by multiplying the most reliable figures available for the production of oil by the factor 1.2.

Census reported the production of both tung meal and oil for the years 1950-54. From these statistics, it can be calculated that the ratio of the weight of meal to oil produced is 1.2. The production of tung meal for the years 1945-54 for the principal tung-producing countries is given in Table IV. Except for the United States for the years 1950-54, the production figures were calculated by multiplying the most reliable figures obtainable for oil production by the factor 1.2.

Actually, the ratio of meal to oil varies from mill to mill and even more from country to country, depending primarily on the amount of shell left with the kernels after hulling. In China, since only the hulls are removed by hand, and not the shells, the ratio of meal to oil produced is higher, probably about 2.0, with a lower nitrogen content in the meal (2 to 3%).

3. Appearance and Structure

The tung tree belongs to the Spurge family, the tung oil or wood oil of commerce being produced from the seeds of *Aleurites fordii* and *Aleurites montana*. In China, where the trees are native, tung oil is produced from both species. In the United States and in Argentina the

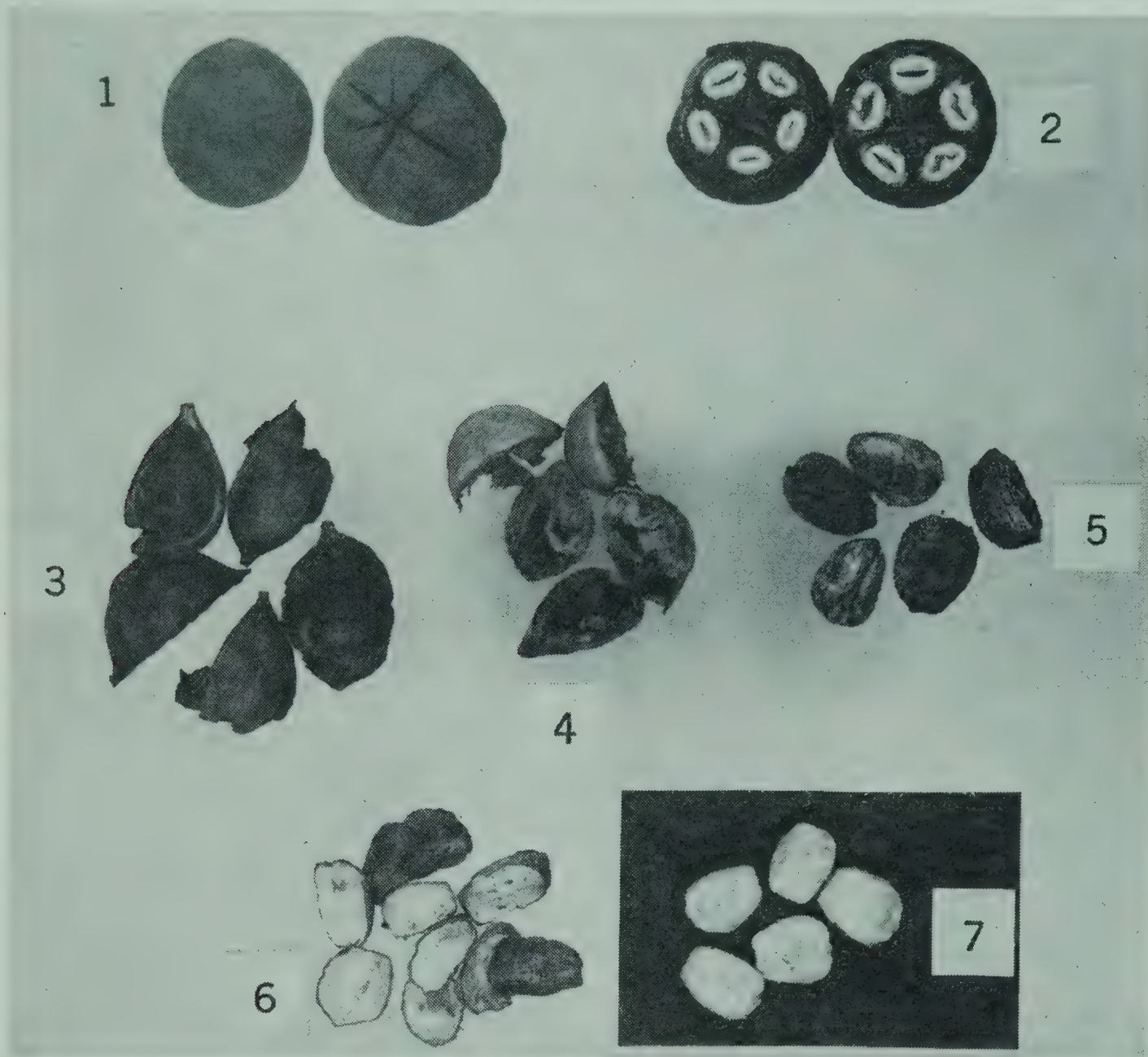


FIG. 2. Tung fruit and its components. The fruit (1) consists of four or five seeds (5), each of which is enclosed by a thin inner hull (4), and the whole surrounded by a fibrous outer hull (3) about $\frac{1}{4}$ inch thick. Cross sections (2) show the arrangement of the seeds in the fruit. The seeds consist of kernels (7) surrounded by a thin hard shell (6).

oil is produced solely from *A. fordii*. There are other species of *Aleurites* which produce oil in commercial quantities (lumbang, bagilumbang, and Japanese tung oils), but these meals will not be discussed further. For the most part they are similar to that from *A. fordii*. Hawaiians used the roasted kernels of *A. moluccana* (lumbang oil) in feasts but could eat them only in small quantities because of their toxicity. It is of interest to note that the tung tree belongs to the same family as the castor and croton plants. All three of these plants produce unusual oils and highly toxic seeds.

The tung fruit (see Fig. 2) consists of four or five seeds about an inch long, each of which is enclosed in a thin inner hull, the whole being surrounded by a fibrous outer hull about $\frac{1}{4}$ inch thick. Each seed consists of a kernel enclosed in a thin hard shell.

The percentage of the different components of the dry tung fruit is shown in Table V.

TABLE V
COMPONENTS OF TUNG FRUIT^a

Component	Content ^b
	%
Kernels	33
Shell	21
Inner hull	7
Outer hull	39
Oil	22
Oil in kernels	66

^a According to R. L. Holmes and R. S. McKinney, *U. S. Dept. Agr. Bur. Agr. and Ind. Chem. Mimeographed Circ. Ser. AIC 357*, 13 pp. (1953).

^b Expressed as percentage of moisture-free material.

4. Processing

In China, the fruit is usually put in piles and wetted down so as to soften the hulls. The seeds are then removed by hand, dried in specially constructed ovens, and ground in stone-edge runner mills. These crushed seeds are molded into cakes which are held in shape by iron bands and pressed in crude wooden presses consisting of a trough cut into a large log. The meal is pressed by driving wedges between one end of the trough and a movable plunger. It is usually pressed more than one time. In 1946 it was estimated (103) that in Szechwan Province alone there were about 30,000 of these primitive presses in operation.

In the United States continuous screw presses have been used from the start of the industry. Solvent extraction has been used in several plants to recover oil from the filter press cake, and in 1954 one mill installed a combination of low-pressure screw press (prepress) and solvent extraction in which all the ground meal is extracted after pressing.

In Argentina, screw presses are used primarily, but there are some mills which prepress followed by solvent extraction.*

The usual processing operation in the United States is as follows: The fruit is picked up from the ground by hand and hulled in disk hullers which remove most of the outer hull but leave a varying proportion of the shell with the

* R. C. Antonissen, private communication.

kernels. If the shelled seeds are not already dry enough (about 10% moisture), they are dried in vertical louvered or bin dryers, and ground to a coarse meal in disk mills. The ground seeds are dried further to 5 to 7% moisture as they are passed through steam-jacketed conveyors and then are put through the screw presses. After filtering through plate and frame filter presses, the oil is ready for the market. The press cake is usually ground through a hammer mill to a fine meal.

5. Composition of Meal

Since half or more of the shell remains with the hulled kernels, the meal consists of the oilseed meats left after the oil is extracted, together with shell fragments. The average composition of the meal in the

TABLE VI
COMPOSITION OF COMMERCIAL TUNG MEAL^{a-b}

Component	Content ^c
	%
Oil	6.1
Crude fiber	42.9
Pentosans	11.3
Nitrogen	4.0
Ash	5.4
Phosphoric acid	1.3
Potash	2.7

^a According to R. L. Holmes and R. S. McKinney, *U. S. Dept. Agr. Bur. Agr. and Ind. Chem. Mimeographed Circ. Ser. AIC 357*, 13 pp. (1953).

^b Average of twenty-five samples from mills in the United States.

^c Expressed as percentage of moisture-free material.

United States, computed from twenty-five samples representing all the mills in the country, is given in Table VI.

Since the average nitrogen content of the oil-free and shell-free kernel is about 7.4% (107) and the average nitrogen content of commercial meal in the United States is 4.0% (108), nearly one-half of the weight of the meal is shell.

The amino acid contents of meal from hand-cleaned kernels and of commercial meal are given in Table VII.

6. Toxicity

Considerable effort has been directed to a study of the toxicity of tung meal in an attempt to develop a process for detoxifying it. In 1953 a patent (109) was issued to Ulrey on a process for detoxifying meal by ammoniation. No commercially feasible process seems to have been

TABLE VII
ESSENTIAL AMINO ACID CONTENT OF TUNG MEAL

Amino acid	Commercial meal ^a		Commercial meal ^b		Meal from clean ^a kernels	
	%	g./16 g. N	%	g./16 g. N	%	g./16 g. N
Protein	24.5	—	20.9	—	46.8	—
Arginine	2.1	9.0	2.3	10.9	4.7	10.5
Histidine	0.4	1.8	0.5	2.3	0.8	1.9
Isoleucine	1.2	4.7	1.0	4.9	2.1	4.4
Leucine	1.5	7.5	1.6	7.6	3.0	8.0
Lysine	1.1	4.6	0.9	4.2	2.2	4.7
Methionine	0.4	1.6	0.5	2.2	1.0	2.0
Phenylalanine	1.0	4.1	1.5	7.3	2.1	4.6
Threonine	0.4	4.1	1.0	4.6	0.9	4.2
Valine	1.5	7.1	1.7	8.3	3.0	7.7

^a According to R. W. Carrol, Quaker Oats Co., private communication.
^b According to C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).

developed, however; at least, no plants for detoxifying meal have been in operation in the United States.

Rusoff and associates (110) fed to 12-day-old chicks solvent-extracted press cake which has been freed of solvent by steaming at 330°F. and stored for a considerable length of time. They observed no definite signs of toxicity when the meal amounted to as much as 20% of the rations, although the chicks on rations containing the cake consumed less and gained less in weight. Erickson and Brown (111) found oil-free tung kernels to be highly toxic to rats, but the oil was non-toxic. The meal could be detoxified by heating with steam for 2 hours at 230°F. Extraction with alcohol destroyed the toxicity of the meal, but the alcohol extract itself was not found to be toxic to rats.

From chemical and biological tests on impure extracts Emmel (112) concluded that a toxic saponin was present in the leaves and kernels of tung; its toxic character could be destroyed by hydrolysis with 5% hydrochloric acid. A second toxic principle was also present which could be removed by extraction with ethyl alcohol.

Davis and associates (113) found that tung meal autoclaved at 128° and 22 pounds per square inch pressure was not safe to use in chicken feeds. Bryan (114) inferred that the toxic principle was a toxalbumin and claimed a method for detoxifying the meal, but the details of the method have never been published.

Lee and Watson at Louisiana State University (115-117) reported that the toxicity of tung meal from screw presses decreased markedly on storage—but the toxicity of meal prepared by extraction in the laboratory did not so decrease. They found that meal could be detoxified by a combination of steaming and alcohol extraction but concluded that the protein in the detoxified meal had little value as a supplement to cereal proteins. Lee (118) later found that addition of lysine to the detoxified meal gave superior results as compared

to the meal alone. Their evidence indicated the presence of two toxic substances. One was soluble in alcohol and was heat-stable under conditions unfavorable to oxidation, but was easily destroyed by the ferric ion and by saponification with alcoholic potash. The other toxic substance was insoluble in alcohol and easily destroyed by moist heat. Watson (117), by liquid-liquid extraction and chromatography, isolated a more highly toxic fraction from the alcohol extract. This material was not identified but did give a negative test for saponins.

Numerous cases of poisoning of human beings in the tung area of the United States have been reported (119-121). No fatalities occurred, but the victims were made very sick. Since the symptoms were similar to those of poisoning by certain alkaloids, Balthrop and associates (119) attempted to detect the presence of an alkaloid but without success. They considered that the main toxic substance was a protein, but also found a glucoside present. They did not determine whether it was toxic. Mann *et al.* (107) studied the toxicity of tung meal, with rats as test animals. They were able to detoxify commercial meal by a combination of steaming and alcohol extraction, but the same treatment did not completely detoxify meal prepared in the laboratory by hexane extraction.

Thus, as this review indicates, the nature of the toxic principle, or principles, in the meal has not been settled, and no commercially feasible process for detoxifying the meal has been worked out as yet. There are probably two toxic substances in tung meal, as Emmel and Lee and Watson claimed. One of them is probably a protein. The other is extractable by alcohol and other organic solvents (but not by petroleum ether) and is comparatively heat-stable. Neither toxic substance has been isolated in a pure form and identified. Watson found the alcohol-soluble principle unstable under oxidizing conditions, and indicated that the toxicity of the fractions decreased as he worked with them. It is also known that the toxicity of commercial tung meal decreases on storage. These facts indicate that the alcohol-soluble toxic material may be unstable to storage.

7. Uses

Since tung meal contains about 25% crude protein, it should be a good stock feed, except for its aforementioned toxicity. Its amino acid content compares favorably with oilseed meals which are used for feedstuffs.

Because of its toxicity, tung meal is used only as a fertilizer, but little experimental work has been published on this application (115). Like all nitrogenous oilseed meals it is a good source of organic nitrogen and is especially valuable for sandy soils because its nitrogen cannot be leached from the soil as rapidly as mineral nitrogen. For this reason, much of the tung meal produced in the United States is used in the orange groves on the sandy soils of Florida.

At a price of 25 cents per pound for oil, a ton of tung fruit is worth about \$80.00 based on the oil alone. From a ton of tung fruit approximately 384 pounds of meal is obtained, worth \$2.07 (at \$16.00 per ton). The hulls are of such little value that it costs about as much to grind them as they are worth, with the result that most of the hulls are simply burned. The ratio of the value of the oil to that of the meal in tung is about 40 to 1 as compared to a ratio of less than 2 to 1 for cottonseed in the United States. This ratio would decrease if the value of the meal could be increased by some method of detoxification.

8. Trends

Tung oil, like all vegetable drying oils, is meeting competition from various synthetics. A survey (122) of drying oils in protective and decorative coatings showed that the use of synthetic materials is steadily increasing as compared to the amount of vegetable oils employed. Declining markets and unfavorable prices for tung oil consequently have decreased the rate of new commercial plantings in the United States and South America. Tung, as a perennial crop that requires several years to come into production, is at a disadvantage in comparison with the annual oilseed crops because its production cannot be varied from one year to the next depending on the demand for the oil.

The future growth of the tung oil industry in the United States and South America depends on a favorable price for tung oil. In the United States higher yielding varieties have been developed, and in some instances older plantations of unimproved varieties of tung already are being destroyed and replaced by these new varieties. If favorable prices were to prevail, the production of tung oil in the United States could be increased substantially, but it remains to be seen just how intensive the competition from synthetics and other vegetable oils will become.

An economical process for detoxifying tung meal would strengthen the competitive position of the tung industry.

IV. ACEITUNO MEAL

Aceituno fat is produced from the seed of *Simarouba glauca*, a tree native to central and northern South America. Its common name (aceituno) is the Spanish word for olive, which it resembles in appearance. The fruit is about 37 mm. long by 20 mm. in diameter and contains a sweetish pulp surrounding the seed which is relished by children. The seed is about 20 mm. by 12 mm. and consists of 30% kernels and 70% shell. The fat content of the kernel varies from 55 to 65% (123).

So far, all production of aceituno fat has been from seeds gathered by natives from wild trees. Plantations of trees have been set out but

have not as yet come into production (124).^{*} The only factory in Salvador processing aceituno purchases 800,000 to 1,200,000 pounds of kernels per year. The meal produced is probably about half the weight of the kernels.

The fat is a solid at ordinary temperatures and is used mostly as a shortening in human food. The meal is toxic and cannot be used for food; consequently it serves only as a fertilizer. It contains about 8% nitrogen, 2% phosphoric acid, 1% potash, and 8 to 12% oil (123).^{*} Nothing is known concerning the toxic principle in aceituno seed. It can be extracted from ground seeds by water or wet butyl alcohol. During attempts to purify the material further, it lost its toxicity.^{*} A bitter principle, glaucarubidin, has been isolated from aceituno seed, but this is relatively non-toxic (125).^{*} A glycoside, simaroubidin, which has promise as an amebicide, has been isolated from the bark of another species of *Simarouba* (126).

No doubt a certain amount of aceituno fat will continue to be produced from wild trees, but the amount that may be grown on plantations will depend on how successful the young groves already planted prove to be.

V. OTHER TOXIC MEALS

There are various other toxic oilseed meals but most of them are of little importance commercially. Most of the following information was taken from Jamieson (127).

The meal from apricot kernels, as well as prune, peach, plum, and cherry, all contain the glucoside amygdalin. Although amygdalin is not toxic, it is easily hydrolyzed to yield highly toxic hydrogen cyanide. These meals are not used as feedstuffs without first being treated to remove the amygdalin.

Physic nut (*Jatropha curcas*) meal, which is produced in small quantities in tropical America, is also toxic and can be used only for a fertilizer. The toxic substance is supposed to be curcin, a toxalbumin. The oil is used as a purgative and for soap-making.

Other toxic meals from oils which are produced in commercial quantities are andiroba (*Carapa* sps.), ben (*Meringa* sps.) and croton (*C. tiglium*). Meals from *Madhuca* sps. and *Palaquium* sps. are believed to contain saponins and are not suitable for cattle feeds. Species of *Madhuca* and *Palaquium* are loosely called illipe, although this name is applicable only to *Shorea stenoptera*, the Borneo illipe nut. Meal from this nut is not toxic. (See also Chapter 22.)

^{*} M. A. Jones, J. A. Fuentes, and J. Boyer, Servicio Cooperativo Interamericano de Agricultura, Guatemala, unpublished manuscript.

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CHAPTER 32

PLANT RESIDUES AND POMACES

E. G. KELLEY

I. INTRODUCTION

The value of plant residues and pomaces as a source of protein meals is largely dependent on economic needs. In periods of high production and surpluses in any particular country it is difficult to justify the economic production of many of the possible products that can be made from the so-called wastes of the fruit and vegetable industries. On an international scale, however, the need for additional protein is always present, and production is never adequate. As a consequence, waste plant residues and pomaces are rarely found in many countries. Of course, as large-scale processing industries develop in more areas of the world, residues are bound to occur.

Just what is meant by the terms plant residues and pomaces? Whenever a fruit or vegetable is harvested for use as a human food or drink, only a part of the product is utilized. Often the quantity of the unused portion exceeds that of the usable portion. Furthermore, the so-called waste not only exceeds the food portion of the plant in quantity, it often contains a much greater amount of protein. These are the plant residues which will be discussed in this chapter. There will also be mention of the residues of coffee and cocoa.

II. SOURCES OF VEGETABLE RESIDUES AND POMACES

1. Vegetable Wastes

a. Field

Many vegetable crops are harvested from the fields in nearly marketable conditions by machines especially equipped to perform digging and cutting operations. There has been a continued trend toward the use of these machines in the United States in the last ten years to min-

imize labor requirements. As a consequence, crops like beets, carrots, turnips, and parsnips come into the packing sheds with little waste. Instead the leaf and stem wastes or residues remain spread on the ground where they are allowed to wilt down to be plowed under as humus. A serious objection to this practice is that it tends to spread diseases or retain them in the same field for years. Rotation of crops is often practiced to combat the field-borne diseases, but many growers feel that harvesting of the whole plant with separation at a central packing shed would be preferable, if the waste portions could be utilized. Cover crops could be substituted as a source of humus.

b. Viner Stations

Green pea and lima bean wastes occur in large quantities at viner stations which are located in centralized areas of the fields. In good dairy areas these viner wastes are ensiled and used as feed. In locations where they cannot be sold for feed the wastes are either spread on fields or allowed to rot in piles for later use as humus. Under these conditions they present serious problems because of odor and ground contamination.

c. Processing Plants

Wastes from processing plants occur in the form of trimmings, peels, seeds, bean snips, and extractives from washing and blanching operations. Solid residues are collected by belts or by screening of wash waters and are usually carried by truck to disposal dumps or distributed in small piles as humus on adjacent fields. In this form they constitute a serious health menace in the neighboring communities. The wash and blanch water extractives present an even more serious disposal problem; it has been customary to flush this water into streams or rivers or into sewers if the plant is located in urban areas. Government controls are becoming increasingly rigid, and other methods of disposal are being required.

d. Tomato Pomace

One of the major processing vegetables in the United States is the tomato; some 3¼ million tons of tomatoes were produced for processing in 1953. Some of the tomatoes were canned, but by far the largest quantity went into making of juice and juice products, i.e., catsup, paste, and purée. The residue from these operations consists of skins, seeds, trimmings, and liquor, or tailings as they are called in the industry. Their disposal has consisted primarily in dumping of solids in remote areas

and flushing of liquid effluents into sewage plants or into streams and rivers, and has presented difficulties.

e. Quantity of Wastes

The quantity of vegetable wastes available from the above-mentioned sources cannot be estimated accurately. In 1955 the production of vegetables in the United States for the fresh market was 10,289,800 tons, that for processing 6,142,900 tons, or a total of 16,432,700 tons. Morris *et al.* (1) and Morris (2) estimated waste in the United States from commercial vegetables producing primarily leafy wastes for the year 1944 as follows: Total production of sixteen vegetables for fresh market and processing was 3,401,398 tons. On the basis of information obtained from fresh market packing plants, processing plants, and trade organizations and publications, the total waste connected with the production of these sixteen vegetables was estimated to be 4,528,040 tons. If the same factor of 1.33 (tonnage of waste/tonnage of edible vegetable) were applied to the total estimated tonnage of vegetables produced in the United States in 1955, the waste from this production would amount to 21,855,491 tons. With an average moisture content of 88%, the dry matter in this tonnage of waste would be 2,622,658 tons; and with an average content of crude protein of 15% on a dry weight basis, the protein in the total production of vegetable wastes would amount to about 393,398 tons.

2. Citrus Residues

Citrus fruits, consisting primarily of oranges, grapefruit, lemons, limes, and tangerines, have become, within the last thirty years, one of the great agricultural commodities. The production of these five citrus fruits for 1953 in the United States was given as 194,990,000 boxes, with the weights per box ranging from 65 to 90 pounds. With an average weight of 80 pounds per box, this would amount to a production of 7.8 million tons of fruit in the United States.

In 1951 Owens *et al.* (3) stated that more than 2 million tons of citrus pulp, peel, and rag remained each year after citrus fruits were processed into juice, frozen concentrate, and sections. This waste material, like vegetable wastes, was formerly disposed of by spreading the solid waste on adjacent lands and flushing the liquid wastes in ponds, streams, lakes, or sewers. This procedure became so offensive that Government agencies found it necessary to develop other methods of disposal. As a result, 80 to 90% of citrus wastes are converted to useful products.

3. Coffee and Cocoa Residues*

a. Coffee

Of a total consumption of about 2 billion pounds of coffee beans in the United States, about 20% is converted into soluble coffee. This amounts to 200,000 tons of green beans or 190,000 tons of roasted coffee beans. In the process of making soluble coffee, 25 to 40% of the roasted bean is extracted, leaving 120,000 to 150,000 tons of residue on the dry weight basis.

b. Cocoa

The by-products obtained from the cocoa and chocolate manufacturing industry are cocoa shells and cocoa germs. On a world-wide basis, about 720,000 tons of cocoa beans are used annually in the manufacture of these two products. These provide about 70,000 tons of cocoa shells and 7000 tons of germs. The shells contain from $\frac{1}{2}$ to 1% of the very valuable cocoa nibs, and the germs usually contain from 10 to 25% of cocoa nibs after mechanical separation.

III. RECOVERY OF VEGETABLE WASTE MATERIALS

1. Vegetable Leaf Meals

A survey by the staff of the Eastern Regional Research Laboratory of the U.S. Department of Agriculture of eighty-three vegetable tissues for protein and vitamins indicated that certain of these tissues were sufficiently high in nutritive value and in content of potential industrial chemicals to warrant more intensive study. Table I shows the composition of many of the wastes. The leafy tissues of wastes from beets, broccoli, lettuce, lima beans, and peas are the most promising, since they occur in large amounts and have a high enough protein content. Other wastes could be used to supplement the supplies of total waste for a processing plant, but these would not support a commercial operation by themselves.

The major problems of vegetable waste utilization are to find a way to process the plant material as soon as possible after harvest and to work out a method for separation of the more valuable leafy tissue from the more fibrous stemmy portions. Dehydration in commercial-type dryers is the only practical method of preserving fresh vegetable tissues to obtain high-quality protein meals. This may be accomplished by a process of fractional drying in which the fresh material is dried in a high-velocity stream of air at temperatures ranging from 250° to

* The material on these commodities was largely provided by Dr. A. Kentie, Technical Director, The Nestlé Company, Inc., Fulton, New York.

TABLE I
COMPOSITION OF TYPICAL DRIED VEGETABLE TISSUES

Vegetable tissue fraction	Moisture	Proportion of whole top		Chemical analyses ^a (moisture-free basis)				
		Fresh basis	Dry basis	Crude ^b protein	Crude fiber	Ether extract	Carotene	Riboflavin
	%	%	%	%	%	%	p.p.m.	p.p.m.
Beet								
Leaf	90.4	52.6	54.2	27.3	6.0	6.2	568	21.5
Stem	91.0	47.4	45.0	13.6	14.7	1.7	48	7.4
Broccoli								
Leaf	81.5	36.4	53.7	35.9	7.6	8.5	803	25.6
Petiole and stem	91.0	63.6	46.3	19.0	16.5	3.3	81	8.5
Cabbage, leaf	—	—	—	22.4	8.2	4.7	295	9.9
Carrot								
Leaf	79.0	51.2	65.1	27.9	10.1	5.6	295	15.7
Stem	88.1	48.8	34.9	11.1	19.3	5.4	41	8.1
Cauliflower								
Leaf	—	—	—	26.6	9.5	4.1	185	23.2
Petiole	—	—	—	17.1	17.3	—	28	9.2
Celery								
Leaf	87.0	—	—	27.2	3.5	6.9	352	18.4
Stalk	94.0	—	—	12.6	14.3	3.1	11	5.7
Collard								
Leaf	—	—	—	27.3	6.8	5.3	251	15.8
Petiole	—	—	—	14.8	9.8	—	28	—
Corn, sweet, leaf	—	—	—	17.1	26.6	5.5	578	5.5
Kale								
Leaf	77.3	57.5	63.3	29.4	7.6	5.8	340	21.0
Petiole and stem	82.3	42.5	36.7	16.2	10.0	4.2	21	8.0
Lima								
Bean	61.6	20.8	28.1	23.9	6.0	3.7	3	2.4
Leaf	67.7	15.8	18.0	19.4	10.5	6.4	465	12.4
Pod	77.1	25.8	20.9	10.0	37.8	3.0	14	3.7
Stem	74.3	36.9	33.5	9.2	40.1	2.2	36	3.9
Parsnip								
Leaf	—	—	—	22.9	8.0	5.0	232	11.9
Stem	—	—	—	6.0	17.2	—	4	4.3
Pea								
Leaf	65.7	12.3	14.1	21.7	14.4	5.8	346	26.2
Pea	80.1	22.4	19.9	28.8	9.2	1.7	4	7.8
Pod	84.0	35.5	25.2	14.1	18.4	1.2	23	7.8
Stem	67.4	29.9	41.0	11.0	39.2	2.3	47	9.6
Rutabaga								
Leaf	82.2	36.1	51.4	31.5	6.3	6.5	257	20.9
Stem	90.5	63.6	48.4	18.5	14.9	—	13	8.5
Turnip								
Leaf	87.3	46.8	61.9	30.9	7.5	4.4	473	20.3
Stem	93.1	53.2	38.2	18.0	10.3	—	54	11.6
Spinach								
Leaf	90.4	45.1	54.7	32.0	6.8	4.1	314	14.6
Stem	93.5	55.0	45.3	22.5	9.3	—	120	8.5

^a E. G. Kelley, *Yearbook Agr. U.S. Dept. Agr.* 843 (1950-1951).

^b Nitrogen \times 6.25.

300°F. The thin leaf blades dry more rapidly than the thicker petiole and stem parts of the waste; the dry leaf blade is brittle, and when it is subjected to breaking and screening action it can be separated from the partly wet and tougher stemmy material (4).

Table II shows the yield and composition of six leaf meals prepared from vegetable wastes (5).

More detailed information on the yield and composition of fifteen leaf wastes together with details of drying procedures using both tray and continuous-belt dryers can be obtained by reference to the work

TABLE II
AVERAGE YIELD AND COMPOSITION OF LEAF MEALS FROM VEGETABLE WASTES^a

Vegetable waste	Mois- ture	Yield ^c	Composition (dry basis) ^b				
			Crude ^d protein	Crude fiber	Ether extract	Carotene	Ribo- flavin
	%	%	%	%	%	p.p.m.	p.p.m.
Beet tops	92.0	5.6	29.6	6.2	7.6	460	18.4
Broccoli	88.5	6.6	35.7	6.1	9.5	460	24.7
Carrot tops	80.7	9.6	18.0	8.5	5.1	158	10.1
Lima bean leaves	73.8	17.8	21.2	6.9	6.0	297	14.0
Pea vines	81.5	9.7	14.6	18.8	4.1	85	16.8
Rhubarb	88.6	10.7	27.4	6.8	—	285	7.0

^a D. A. Colker and R. K. Eskew, *U.S. Dept. Agr., Bur. Agr. and Ind. Chem. AIC-76*, 4 (1945).
^b E. G. Kelley, *Yearbook Agr. U.S. Dept. Agr.* 843 (1950-1951).
^c On the basis of fresh material.
^d Nitrogen \times 6.25.

of Kelley (6). The use of rotary alfalfa dryers for the fractional drying of vegetable wastes has been described by Aceto *et al.* (7).

Feeding studies on poultry were carried out with the vegetable leaf meals in which they were used as a source of carotene (vitamin A) or riboflavin rather than as a protein source (8). Only in one experiment with solvent-extracted broccoli leaf meal was the meal used as the source of the protein in broiler diets (9). In this particular feeding trial, growth was maintained on the broccoli meal, but the addition of soybean oil meal to the leaf meal produced more rapid growth.

The quantitative determination by microbiological methods of the content of the ten essential amino acids in vegetable leaf meals revealed that most of the leaf meals were similar in over-all composition and that they contained the essential amino acids (10). (See Table III.) Determination of methionine in the leaf meals required special

TABLE III
AMINO ACIDS IN LEAF MEALS^a

Source	Crude protein	Histidine	Arginine	Lysine	Leucine	Iso-leucine	Valine	Methionine ^b	Threonine	Phenylalanine	Tryptophan
	%	%	%	%	%	%	%	%	%	%	%
Beet	24.3	1.3	4.1	5.4	6.4	4.2	5.1	1.7	3.8	5.8	1.2
Broccoli	41.0	1.5	4.8	4.5	6.4	3.2	4.5	1.8	3.3	6.0	1.4
Carrot	19.6	1.9	4.3	4.5	7.1	4.5	5.5	1.7	4.4	6.5	1.4
Celery	23.2	1.5	4.0	2.4	6.8	3.9	4.8	2.2	3.4	4.5	1.3
Corn	19.4	1.3	3.9	3.2	6.9	3.6	4.8	2.8	3.3	5.4	1.3
Kale	24.7	1.6	5.1	3.1	6.5	3.4	4.6	0.9	3.5	4.4	1.1
Lima bean	16.9	1.3	4.2	3.6	6.6	3.6	5.0	1.2	4.0	7.0	1.4
Pea	23.6	1.6	4.6	4.9	7.8	4.4	5.7	1.0	4.4	6.0	1.5
Rhubarb	26.1	1.9	4.7	5.4	8.4	4.0	5.3	1.0	4.0	6.1	1.6
Spinach	25.7	1.3	4.4	4.7	6.8	3.6	5.0	2.3	3.9	4.7	1.1
Turnip	23.9	1.4	4.5	3.0	6.8	3.9	4.8	2.2	4.0	5.3	1.3
Range	16.9-41.0	1.2-3.9	3.9-5.2	2.4-5.4	6.4-8.4	3.2-4.6	4.5-5.8	0.9-2.8	3.3-4.5	4.4-7.0	1.1-1.6
Other leaf meals ^c											
Alfalfa	18.1-19.4	1.2-2.1	3.1-4.3	3.6-4.9	6.2-6.6	3.6-5.2	4.1-4.4	0.2-1.4	2.2-3.6	4.1-4.6	1.0-1.4
Grass	19.4	3.1	6.7	7.2	13.4	9.3	10.3	2.1	6.7	8.8	2.1
Ryegrass	12.5	2.2	5.4	3.3	6.2	4.0	5.0	1.1	3.9	3.0	1.3

^a E. G. Kelley and R. R. Baum, *J. Agr. Food Chem.* 1, 680 (1953); calculated on the basis of 16% nitrogen.

^b Two-hour hydrolysis.

^c R. J. Block and D. Bolling, "Amino Acid Composition of Proteins and Foods," 2nd ed. Charles C Thomas, Springfield, Illinois, 1951.

TABLE IV
AMINO ACIDS IN PROTEIN CONCENTRATES^{a, b}

Source	Leaf protoplasts									
	Crude protein %	Histidine %	Arginine %	Lysine %	Leucine %	Isoleucine %	Valine %	Methionine %	Threonine %	Tryptophan %
Beet	40.9	1.9	5.9	5.6	8.6	5.5	6.3	1.6	4.9	1.7
Broccoli, fat-free	78.2	1.8	5.2	5.3	8.9	4.7	5.9	1.8	4.4	2.3
Carrot	27.0	2.0	6.9	5.5	10.7	6.3	7.2	1.8	6.1	2.2
Lima bean	48.4	1.5	5.5	3.8	8.0	4.7	5.6	1.2	4.6	1.7

Leaf proteins extracted with formic acid									
Source	Histidine %	Arginine %	Lysine %	Leucine %	Isoleucine %	Valine %	Methionine %	Threonine %	Phenylalanine %
Broccoli	2.2	6.0	5.5	9.2	5.3	6.4	2.0	5.0	7.5
Carrot	2.1	5.7	5.4	10.6	5.5	7.0	3.4	5.0	7.7
Lima bean	1.9	5.7	5.2	9.1	5.2	5.9	2.2	4.7	7.0
Pea vine	1.8	5.4	5.8	8.7	5.1	6.1	1.8	4.5	6.7
Rhubarb	2.4	6.3	5.4	9.8	5.0	6.6	2.1	4.6	6.9
Rutabaga	1.7	5.4	5.6	7.2	4.7	6.0	1.6	4.2	5.8
Spinach	1.8	5.4	5.8	8.2	4.2	6.0	1.8	4.6	5.8

^a E. G. Kelley and R. R. Baum, *J. Agr. Food Chem.* 1, 680 (1953).

^b Calculated on the basis of 16% nitrogen.

hydrolysis conditions, since the presence of carbohydrates caused some losses in the normal 10-hour acid hydrolysis. A two-hour hydrolysis gave more satisfactory results.

Partially purified proteins were made from vegetable leaves by a fermentation procedure and also by formic acid extraction. Table IV shows the protein and amino acid contents for some of the vegetable leaf protein concentrates. In Table V the amino acid contents of the meals and the protein concentrates are compared. The amino acid content of the leaf meals is about 25% lower than in the protein concentrates when the comparison is made on the basis of total nitrogen

TABLE V
COMPARISON OF AVERAGE AMINO ACID CONTENTS OF THREE LEAF MEALS
AND THEIR PROTEIN CONCENTRATES^a

Leaf preparation ^b	Histidine	Arginine	Ly-sine	Leucine	Iso-leucine	Valine	Methionine	Threonine	Phenylalanine	Tryptophan
	%	%	%	%	%	%	%	%	%	%
Meals	1.4	4.4	4.2	6.7	3.8	5.0	0.48	3.9	6.5	1.4
Protoplasts	1.8	5.9	4.9	9.2	5.2	6.2	1.6	5.0	7.9	2.1
Formic acid extract	2.1	5.8	5.4	9.6	5.3	6.4	2.5	4.9	7.4	—

^a E. G. Kelley and R. R. Baum, *J. Agr. Food Chem.* **1**, 680 (1953); calculated on the basis of 16% nitrogen.

^b Broccoli, carrot, and lima bean.

(Kjeldahl N). Recent studies on cabbage leaves have revealed that as much as 50% of the total nitrogen is non-protein nitrogen.*

Although the vegetable leaf meals are good sources of protein for poultry and livestock, it is doubtful that they could compete with oil-seed meals. They do, however, contain other valuable ingredients which put them on a competitive basis with other leaf meal feed supplements. Carotene (provitamin A), and xanthophyll, a yellow pigment desired in poultry feeds for flesh coloring, are present in considerable amounts, and these can be used either in the form of the dehydrated leaf meal or as solvent-extracted oils. Poultry feeding trials proved that these two substances could be utilized effectively from the leaf meals and that the extracts also made excellent feed supplements. Wall and Kelley determined content of tocopherol (vitamin E) (11) and leaf sterols in various leaf meals (12), and Wall (13) separated a number of fractions of these materials by molecular distillation. Since the leaf meals were prepared from fresh material under carefully controlled temperature

* E. G. Kelley, S. Krulick, R. R. Baum, R. M. Zacharius, and J. J. McGuire, personal communication.

conditions, a high quality chlorophyll salt could be prepared from most of them (14).

Petiole and stem residues remaining after preparation of vegetable leaf meals by the fractional drying process are of lower value, and their economic recovery is uncertain. They can be dried further and ground to provide bulk for feed mixtures, since they often do have appreciable protein content. They also contain sufficient fiber to be used for mulch or litter at a price competitive with those of peat moss or shell products. They are excellent as a source of humus for soil rebuilding.

2. Tomato Pomace

The recovery of tomato pomace has been studied by Edwards *et al.* (15). The process requires additional steps other than the drying

TABLE VI
ANALYSIS OF DRIED TOMATO PRESS CAKE^a

Ingredient	Press cake	Press cake with added concentrate
	%	%
Moisture	8.0	8.0
Crude protein ^b	22.5	21.0
Fat	14.2	9.8
Fiber	29.6	21.9
Ash	3.3	5.9
Nitrogen-free extract	22.4	33.4

^a P. W. Edwards, R. K. Eskew, A. Hoersch, Jr., N. C. Aceto, and C. S. Redfield, *Food Technol.* **6**, 383 (1952).

^b Nitrogen \times 6.25.

described for leafy wastes. Because of their semiliquid state it is necessary first to obtain a high solids fraction from the chopped culls and trimmings by passing them through a cyclone. The tailings from the cyclone are added to similar tailings from juice-making cyclones and pressed in a continuous rotary press to yield a press cake and a dilute liquor. The press cake, of about 63% moisture content, is dried in rotary alfalfa dryers under suitable conditions.

Since the liquid waste from the presses and that from the waste cyclone still contain about 5% solids, these are concentrated by evaporative procedures to about 30% solids. This thick liquid can then be added in certain proportions to the dried press cake and the combined product passed again through the rotary dryer.

Table VI shows the analysis of the dried press cake, alone and plus concentrate.

Dried tomato pomace is made in commercial quantities for use in dog foods and in feeds for fur-bearing animals. It is largely made from the press cake alone because of the lower cost of production. When effluents become a more costly sewage disposal problem they can be incorporated into the dried pomace.

About 2 to 3% of the dried press cake, added to dry-type feeds, is valuable for the prevention of diarrhea in dogs and mink. Broilers utilized dried tomato press cake more efficiently than they did alfalfa meal, and tomato press cake and concentrate were superior as a growth promoter to wheat middlings when used as a replacement at a 5% level. As noted earlier, $3\frac{1}{4}$ million tons of tomatoes were processed in the United States in 1953. For every million tons of tomatoes there would result about 123,800 tons of recoverable waste containing 11,300 tons of solids. There exists a potential for production of about 36,000 tons of dried pomace, but at present (1957) only a fraction of this amount is being made.

3. Citrus Pomace

Von Loesecke has reviewed and summarized much of the recent work on citrus wastes (16). There is also a handbook of the chemistry and technology of the citrus industry (17). Most of the following description of the recovery of various products from citrus wastes has been taken from these two publications. Information on the use of citrus fruit and waste for feeding dairy cattle in Israel is given by Volcani (17a).

In 1949 there was in the United States an estimated $3\frac{1}{2}$ million tons of solid citrus waste and about 4 billion gallons of liquid effluents. Probably about 90% of the solid waste is dried yearly for feeds, and considerable quantities of the liquid effluents are converted to feed, molasses, and other by-products. Solid wastes consist of cannery wastes such as peel, rag (core plus segment membranes), and seed, screening from citrus pulp-drying effluents, sludge from peel oil preparations, residues from plants producing citric acid and pectins, and still slops. The liquid wastes are largely cannery effluents, pulp drying plant and distillery effluents, and effluents from citrus molasses and peel oil plants.

Table VII shows the analysis of several types of solid citrus cannery waste.

The solid wastes have been utilized most successfully for the preparation of dried citrus pulp for cattle and swine feeds. The method of preparation of this dried pulp is essentially the same in the numerous drying plants making this material on a commercial scale. Some varia-

TABLE VII
ANALYSES OF CITRUS CANNERY WASTE^a

Constituent	Florida grapefruit		California grapefruit, peel and rag	Israel grapefruit, peel ^b	Lemon, peel and rag
	Peel	Rag			
	%	%	%	%	%
Total solids	16.71	15.60	22.02	17.9	16.17
Ash	0.74	0.75	0.70	0.70	0.82
Volatile oil	0.43	—	0.56	—	—
Acid, as citric	0.74	0.63	0.43	—	0.60
Crude fiber	1.71	1.44	2.00	1.9	2.73
Crude protein ^c	1.13	1.06	1.63	1.2	1.56
Crude fat (ether extract)	0.28	0.16	0.23	0.3	—
Total sugar (as invert)	6.35	6.30	8.68	—	—
Pentosans	0.83	0.44	1.31	—	2.61
Pectin (calcium pectate)	3.10	3.56	3.93	—	—
Naringin	0.40	0.10	0.63	—	—

^a Except where noted, the data are from H. W. Von Loesecke, *Ind. Eng. Chem.* **44**, 476 (1952).

^b A. Bondi and K. Mayer, *Empire J. Exptl. Agr.* **10**, 93 (1942).

^c Nitrogen \times 6.25.

tions occur in dryer design; both direct-fired and steam tube dryers are in common use. The nature of the presses used in the preliminary removal of water may vary, but in all instances the end result is about the same.

A general description of the method follows: Lime (0.3 to 0.5%) is added to the wet waste; it is then shredded or chopped in a cutting mill and slowly mixed and aged in a pug mill. The lime is added to neutralize the fruit acids and combine with the fruit pectins to form calcium pectate which aids in the pressing operation that follows. The material is pressed in continuous presses as dry as possible and fed to the dryers in a uniform manner. With proper control of drying rate and temperature, a fluffy, light-colored feed is produced in a yield of about 1 ton of feed from 10 tons of waste. This material is bagged and sold under numerous trade labels as dried citrus pulp. In 1955 over 300,000 tons of this product were produced in Florida. Texas and California also produce considerable tonnages. The market value of this product is in excess of 12 million dollars per year.

Table VIII shows a typical analysis of dried grapefruit waste.

Dried citrus pulp is used primarily as a carbohydrate concentrate. It is low in content of crude protein, fiber, and fat and high in content of nitrogen-free extract. Neal *et al.* (18) found a high coefficient of

TABLE VIII
ANALYSIS OF DRIED GRAPEFRUIT WASTE^a

Constituent	Content	Constituent	Content
	%		%
Moisture	7.54	Naringin	1.52
Ash	6.00	Potassium and sodium chlorides	1.79
Protein ^b	6.49	Silica	0.23
Crude fat	5.46	Iron and aluminum (Al ₂ O ₃ +	
Crude fiber	14.06	Fe ₂ O ₃)	1.94
Pentosans	14.35	Magnesium (Mg)	0.34
Sucrose (non-reducing sugars)	9.18	Calcium (Ca)	1.63
Reducing sugars (as invert)	2.82	Phosphates (P ₂ O ₅)	0.26
Total sugars	11.00	Sulfur (S)	0.10
Pectin (alcohol precipitate)	18.20	Chlorides (Cl ₂)	0.05

^a H. W. Von Loesecke, *Ind. Eng. Chem.* **44**, 476 (1952).

^b Nitrogen \times 6.25.

digestibility for the whole dried product amounting to 83 and 81% for grapefruit and orange pulp, respectively; the nitrogen-free extract fraction is from 88 to 92% digestible. The crude protein fraction was lower in digestibility, showing only 24.8 and 36.6% digestible material in the dried grapefruit and orange pulps, respectively. Jones *et al.* (19) showed that a diet containing 25% dried citrus pulp was satisfactory for growth of beef cattle. Arnold *et al.* (20) found the citrus pulp equal to dried beet pulp as a bulky carbohydrate feed for dairy cattle. Kirk and Crown (21) found that the citrus pulp was less satisfactory as a feed for swine, and Mehrhof and Rusoff (22) found that even 5% of the dried grapefruit pulp gave unfavorable results with young poultry, although pullets and laying birds apparently could utilize this feed to a greater extent without affecting their rate of growth or egg production.

Lyman *et al.* (23) have determined the content of crude protein and the ten essential amino acids in a sample of dried citrus pulp. (Table IX.) Townsley *et al.* (24) and Underwood and Rockland (25) have reported on the amino acids of citrus juices and proteins from chromatophores and other solid portions of citrus fruits.

4. Coffee Residues

The main by-product of coffee is the residue which is left after the water-extraction of coffee for the manufacture of soluble coffee. Water-extraction of ground roasted coffee beans removes 25 to 40% of the weight of the roasted coffee bean. This extract is dried and sold as

TABLE IX
AMINO ACID CONTENT OF CITRUS PULP^a

Amino acid	In pulp	In crude protein ^b
	%	%
Arginine	0.28	4.81
Histidine	0.09	1.55
Isoleucine	0.18	3.10
Leucine	0.31	5.33
Lysine	0.20	3.44
Methionine	0.08	1.37
Phenylalanine	0.18	3.09
Threonine	0.18	3.09
Tryptophan	0.06	1.03
Valine	0.25	4.30

^a C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).

^b Calculated on the basis of 16% nitrogen.

TABLE X
COMPOSITION OF COFFEE BEANS AND COFFEE GROUNDS^a

Analysis	Green	Roasted	Coffee grounds
	%	%	%
Moisture	8.75	3.75	5.5
Ash	4.41	4.49	1.0
Fat	12.96	13.76	25.4
Caffeine	1.87	1.81	0.5
Crude fiber	20.70	14.75	38.7
Protein	9.50	12.93	10.59
Water extract	31.11	30.30	—
Nitrogen	—	—	2.4
Lignin	—	—	13.3
Sugar	7.62	1.31	—
Dextrins	0.86	1.31	—
Tannic acid	9.02	—	—

^a Data on all but sugar, dextrins, and tannic acid are taken from W. H. Ukers, "All About Coffee," 2nd ed. Tea and Coffee Trade Journal Co., New York, 1935. The data on the others are from W. L. A. Warnier, *Pharm. Weekblad* **13** (1899).

soluble coffee. The residue, which is saturated with water (about 70%), is difficult to handle without first drying. (The extracted coffee residue is called coffee pulp or coffee grounds by various authors.) Table X gives some data on the composition of coffee beans and coffee residue or grounds. Lyman *et al.* (23) carried out amino acid analyses on dried coffee pulp containing 10.6% crude protein with results as shown in

Table XI. In addition to the possible protein value of the dried coffee residue, it contains about 22% oil and 1 to 2% of a waxy material.

Coffee residue has been used on occasion as a cattle food. Mather and Apgar (26) found that it could be used as a feed for dairy cattle in a concentration as high as 18% (24% when mixed with molasses) with no significant effect on milk production, butter fat percentage, pulse rate or flavor of milk. Body weight of cows on coffee, however, was significantly reduced.

TABLE XI
AMINO ACID CONTENT OF COFFEE PULP^a

Amino acid	In pulp	In crude protein ^b
	%	%
Arginine	0.38	3.68
Histidine	0.21	1.98
Isoleucine	0.40	3.78
Leucine	0.58	5.48
Lysine	0.36	3.40
Methionine	0.28	2.64
Phenylalanine	0.33	3.12
Threonine	0.33	3.12
Tryptophan	0.10	0.94
Valine	0.50	4.72
Crude protein	10.59	

^a C. M. Lyman, K. A. Kuiken, and F. Hale, *J. Agr. Food Chem.* **4**, 1008 (1956).

^b Calculated on the basis of 16% nitrogen.

5. Cocoa Residues

The by-products of cocoa and chocolate manufacture are cocoa shells and cocoa germs. Fermented cocoa beans consist of approximately 88 to 90% cotyledons, 0.7% germ, and between 10 and 11% shell (27).

Processing of cocoa beans involves a separation of the bean constituents into nibs (cotyledons) and shells. The nib portion is further fractionated to remove the germ. It is the germ-free nib which is milled and processed into cocoa and chocolate. Under practical conditions separation is incomplete; hence the shells usually contain ½ to 1% nibs, and the germs, 10 to 25% nibs. Owing to incomplete separation, commercially available cocoa shells contain about 5% or more fat and the germs about 5 to 10% fat. The composition of nibs, shells, and germs is given in Table XII.

Cocoa shells are unsuitable for human consumption because of the high content of fiber; neither are they used to any large extent as a

TABLE XII
COMPOSITION OF COCOA NIBS, SHELLS, AND GERMS

Constituent	Fraction of cocoa bean			
	Nibs ^a	Shells ^{a,b}		Germs ^a
	%	%		%
Moisture	5.0	11.0	3.8	7.0
Fat	53.3	3.0	3.4	3.5
Crude protein ^c	13.4	16.0	14.1	30.5
Tannins	5.8	9.0	5.1	—
Crude fiber	2.6	16.5	18.6	2.9
Ash	2.8	6.5	8.1	6.5
Pentosans	1.5	6.0	7.1	—
Theobromine	1.4	0.7	1.3	3.0

^a Taken from the following: A. Beythien and P. Pannwitz, *Z. Nahr. Genussm.* **46**, 223 (1923); F. Hartel, *ibid.* **47**, 264 (1924); H. Fincke, *ibid.* **50**, 205 (1925).

^b From A. W. Knapp and K. Churchman, *Chem. & Ind. (London)* p. 29 (1937).

^c Nitrogen \times 6.25.

cattle feed. Only part of the nitrogen is available as digestible protein, about 7% of the weight of the shells. Shells contain a considerable amount of vitamin D; it has been claimed that their inclusion in dairy feed increases the vitamin D content of the milk (28).

Some animals, especially horses, seem to be sensitive to the theobromine (3,7-dimethylxanthine) in the shells. In Germany, some deaths were reported of horses, cows, and hogs due to intake of shells. Moreover their ingestion seems to cause constipation; hence the shells are usually mixed with 10 to 15% molasses.

The Ministry of Food and the Ministry of Agriculture in Great Britain issued the following statement in 1943 (29):

Cocoa and chocolate residues including shell are suitable for feeding to adult cattle provided that the daily ration does not exceed 2 lbs. of this material.

In the case of pigs, poultry and calves they are detrimental and the cumulative effect may be serious. Cocoa residues are sold for inclusion in cattle feeds to the extent of 2.5% for feeding to adult cattle only.

Weniger *et al.* state that cocoa shells can be given safely as bulk food to cows in quantities up to 2 or 3 kg. daily (30). In actual practice hardly any is used for feed either in the United States or Europe. Use of cocoa shells as fodder has been reviewed by Sperling (31).

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CHAPTER 33

AMINO ACID COMPOSITION OF FOODSTUFFS

T. H. HOPPER

As has been pointed out repeatedly, the nutritive value of protein foodstuffs is closely associated with the amount of protein they contain and with their content of essential amino acids. Data on amino acid composition are listed throughout this book; they are summarized in this chapter. The protein content of plant products varies greatly, influenced by variety and by soil and climate environment. The amino acid content of protein of a given plant product is considered relatively constant.

In actual practice, the amino acid composition of the foodstuff is what is important. Since, however, the protein content of foodstuffs varies, direct comparison of amino acid composition of various protein sources is not possible unless it is related to their protein content. The ideal procedure for determining protein content would be to isolate the protein from each material. This, however, is difficult to do, and often the results are difficult to interpret. Instead, the protein content of a foodstuff is estimated by multiplying its nitrogen content by an appropriate factor. A list of suggested factors, compiled by Jones, is given in Table I. Despite the differences shown in this table, the generally accepted procedure has been to use one factor (6.25) for all foodstuffs. This has the advantage of simplicity and uniformity; this one factor is used throughout this book. (See discussion on page 17.)

Table II lists the contents of essential amino acids of proteins of selected products of plant, animal, marine, and microorganism origin. Animal and marine products are included for comparison with plant proteins. The values are expressed as grams per 16 grams of nitrogen (conversion factor of 6.25). Nearly all the determinations on which the results are based were made by microbiological methods of analysis. In many instances there is lack of agreement among investigators. There is no basis for averaging values for any one protein, nor is there any criterion for selecting one set of data over another. As time goes on and more determinations are reported, and particularly as chromato-

TABLE I
FACTORS SUGGESTED FOR USE IN CONVERTING PERCENTAGES OF NITROGEN
IN VARIOUS FOODSTUFFS INTO PERCENTAGES OF PROTEIN^a

Foodstuff	Factor
Oilseeds and nuts	
Almonds	5.18
Brazil nut	5.46
Butternut	5.30
Cantaloupe seed	5.30
Castor beans	5.30
Coconut	5.30
Cottonseed	5.30
Flaxseed	5.30
Hazelnut	5.30
Hempseed	5.30
Peanut	5.46
Pumpkin seed	5.30
Sesame seed	5.30
Soybean	5.71
Squash seed	5.30
Sunflower seed	5.30
Walnut	5.30
Cereal grains	
Barley	5.83
Corn (maize)	6.25
Oats	5.83
Rice	5.95
Rye	5.83
Wheat, endosperm	5.70
Wheat, embryo	5.80
Wheat, bran	6.31
Wheat, whole kernel	5.83
Substances of animal origin	
Eggs	6.25
Gelatin	5.55
Meats	6.25
Milk	6.38
Leguminous seeds	
Adsuki beans	6.25
Jack beans	6.25
Lima beans	6.25
Mung beans	6.25
Navy beans	6.25
Velvet beans	6.25

^a D. B. Jones, *U. S. Dept. Agr. Circ.* 183 (1931).

graphic and other newer methods of analysis are applied to the foodstuffs, it will be possible to select the better data. In the meantime, the reader may judge for himself from the range in composition.

For more information the reader is referred to the appropriate chapters in this book and to "Amino Acid Handbook" by Block and Weiss (reference 12 in Table II), and to "Tables of The Amino Acids in Foods and Feedingstuffs," by D. Harvey.*

* Technical Communication No. 19, Commonwealth Agricultural Bureaux, Farnham Royal, Slough, Bucks, England, 1956.

TABLE II
ESSENTIAL AMINO ACID CONTENT OF PROTEINS OF SELECTED PLANT, ANIMAL, AND MICROORGANISM PRODUCTS
(Calculated as grams per 16 grams of nitrogen)

Foodstuff	Argi- nine	Histi- dine	Iso- leucine	Leucine	Lysine	Methi- onine	Phenyl- alanine	Threo- nine	Valine	Tryp- tophan	Tyro- sine	Ref.
Oilseeds, meals, and by-products												
Almond kernels	11.1	1.5	0.4	—	6.6	—	2.4	—	—	1.3	—	1
Babassu meal	12.58	1.71	5.09	6.29	4.19	1.50	4.19	2.91	4.79	0.77	1.93	2
Babassu meal	14.04	1.81	3.87	6.17	4.31	2.33	5.94	3.13	5.24	1.06	—	3
Castor pomace	10.03	1.68	4.65	5.65	3.02	1.46	4.69	3.24	5.40	1.11	—	3
Castor pomace	9.8	1.7	5.2	6.3	2.8	1.5	3.2	3.3	6.8	0.8	—	4
Castor flour	12.2	2.0	5.7	6.3	3.3	1.9	3.5	3.6	6.9	1.1	—	4
Castor pomace	11.0	2.5	5.3	7.2	3.1	1.5	4.2	3.6	6.6	0.8	3.2	5
Castor flour	12.90	2.09	5.32	6.40	3.38	1.78	5.19	3.84	6.74	1.43	—	3
Coconut meal (copra)	11.00	1.55	5.30	6.05	2.60	1.25	3.90	3.10	5.15	0.75	4.00	2
Coconut meal	10.76	1.56	4.72	7.88	2.98	1.51	3.94	2.93	4.85	0.96	1.88	2
Coconut meal	11.43	1.71	4.00	6.30	3.06	1.59	4.23	3.78	5.49	0.94	—	3
Coconut meal	11.2	1.7	4.0	4.9	2.5	1.5	4.3	3.7	5.5	0.88	—	6
Coconut meal	10.4	1.8	3.4	5.4	2.2	1.5	5.1	3.0	5.1	0.99	—	3
Coconut meal	10.3	1.6	3.9	6.7	2.3	1.8	4.1	3.4	6.1	0.9	—	4
Cottonseed meal	9.02	2.50	4.32	6.06	4.65	0.96	5.15	2.86	4.58	1.30	3.04	2
Cottonseed meal	11.02	2.70	4.01	6.20	4.20	1.49	5.25	3.47	4.98	1.59	—	3
Cottonseed flour	11.30	2.57	3.94	6.23	4.16	1.61	5.20	3.36	4.84	1.55	—	3
Hempseed	5.0	3.9	4.4	7.7	2.7	2.2	5.8	3.8	6.3	1.5	—	7
Hempseed protein (edestin)	16.7	2.5	4.7	7.5	2.4	2.4	5.5	3.9	6.5	1.5	—	8

Linseed meal	9.28	1.80	4.57	5.96	3.62	1.66	4.49	3.78	5.55	1.74	—	3
Linseed meal	8.55	1.86	5.92	5.78	4.06	1.00	4.21	3.58	4.92	1.51	2.21	2
Linseed meal	8.63	1.95	4.94	5.86	3.72	1.10	4.30	3.45	4.70	1.19	4.12	2
Palm kernel meal	16.2	1.8	4.6	7.6	3.6	2.7	4.4	3.7	6.2	0.8	—	9
Palm kernel meal	17.2	1.8	5.0	8.3	4.7	2.3	4.8	4.0	6.4	0.8	—	9
Palm kernel meal	13.26	1.62	3.97	6.42	3.44	2.14	4.28	3.13	5.38	1.04	—	3
Peanut meal	10.33	2.16	4.33	6.68	3.53	1.04	4.97	2.98	4.82	1.22	—	3
Peanut meal	11.15	2.18	3.78	6.97	4.35	0.69	5.02	2.79	5.39	0.94	3.69	2
Peanut flour	12.38	2.23	3.89	7.11	4.53	0.71	5.41	2.84	5.50	0.92	3.28	2
Peanut flour	11.26	2.16	4.33	6.74	3.20	1.02	5.08	2.67	4.64	1.28	—	3
Pecan nuts	10.4	3.0	4.9	8.2	5.4	1.7	5.3	4.0	3.5	1.4	—	10
Safflower	7.8	2.0	3.8	5.5	2.7	1.5	5.2	2.9	4.9	1.2	—	4
Safflower	7.78	1.99	3.85	5.52	2.71	1.54	5.25	2.94	4.93	1.18	—	3
Sesame meal	10.04	2.36	3.56	6.12	2.66	2.03	4.48	3.49	4.82	1.22	4.70	2
Sesame meal	11.91	2.21	4.27	6.92	2.76	2.65	4.73	3.64	5.06	1.91	—	3
Sesame seed	8.7	1.5	4.8	7.5	2.8	3.1	8.3	3.6	5.1	1.8	3.5	11
Soybean meal	6.02	2.28	6.37	6.58	6.42	0.67	4.81	3.78	5.00	1.21	3.07	2
Soybean meal	6.04	2.40	6.32	7.80	6.32	0.68	4.77	3.79	4.86	1.33	3.18	2
Soybean meal	7.46	2.49	5.50	7.69	6.17	1.39	4.86	4.03	5.40	1.69	—	3
Soybean meal	7.0	2.5	5.8	7.6	6.6	1.1	4.8	3.9	5.2	1.2	3.2	12
Soybean protein	7.71	2.63	5.59	8.14	6.06	1.33	5.90	3.70	6.58	1.04	3.36	2
Soybean protein	7.20	2.33	5.85	7.77	5.77	1.17	5.53	3.56	5.14	1.46	—	3
Soybeans, Acadian variety	7.75	2.29	5.34	7.75	6.52	1.43	4.95	3.95	5.44	1.51	—	13
Soybeans, Arksey variety	7.56	2.30	5.30	7.86	6.54	1.39	5.11	3.87	5.30	1.45	—	13
Soybeans, A3-176 variety	7.68	2.25	5.34	7.90	6.70	1.43	5.02	3.93	5.43	1.56	—	13

TABLE II (Continued)

Foodstuff	Argi- nine	Histi- dine	Iso- leucine	Leucine	Lysine	Methi- onine	Phenyl- alanine	Threo- nine	Valine	Tryp- tophan	Tyro- sine	Ref.
Oilseeds, meals, and by-products (continued)												
Soybeans, A3K-884 variety	8.09	2.26	5.42	7.85	6.88	1.40	4.87	4.06	5.31	1.50	—	13
Soybeans, A4-107-12 variety	8.01	2.23	5.22	7.93	6.53	1.34	4.84	3.84	5.28	1.48	—	13
Soybeans, Chief variety	7.82	2.30	5.20	7.86	6.59	1.48	5.04	3.96	5.41	1.56	—	13
Soybeans, C.N.S. variety	7.87	2.36	5.19	7.59	6.00	1.31	5.11	3.72	5.35	1.55	—	13
Soybeans, C-463 variety	7.54	2.37	5.39	7.96	6.90	1.45	5.07	3.96	5.40	1.61	—	13
Soybeans, Earlyana variety	7.72	2.29	5.31	7.95	6.74	1.37	5.20	3.98	5.31	1.56	—	13
Soybeans, Gibson variety	7.49	2.30	5.35	8.13	6.91	1.41	5.08	3.82	5.31	1.54	—	13
Soybeans, N-5 variety	7.22	2.16	5.24	7.97	5.97	1.38	5.04	3.58	5.22	1.54	—	13
Soybeans, Lincoln variety	7.53	2.29	5.32	8.08	6.67	1.40	5.22	3.91	5.42	1.64	—	13
Soybeans, Lincoln No. 3 variety	7.72	2.33	5.43	8.45	6.73	1.53	5.17	4.03	5.48	1.60	—	13
Soybeans, Manloxi variety	7.96	2.40	5.53	7.94	7.07	1.50	5.23	3.86	5.34	1.44	—	13
Soybeans, N44-92 variety	7.60	2.49	5.29	8.17	6.70	1.35	5.17	4.04	5.47	1.49	—	13
Soybeans, N44-774 variety	7.85	2.37	5.28	8.04	6.73	1.35	5.13	3.99	5.17	1.57	—	13
Soybeans, Ogden variety	7.49	2.34	5.48	8.16	6.76	1.28	5.31	3.83	5.31	1.42	—	13
Soybeans, Richland variety	8.30	2.35	5.18	7.98	6.47	1.37	4.80	3.84	5.20	1.57	—	13
Soybeans, Roanoke variety	7.64	2.47	5.15	8.02	6.48	1.41	5.23	3.88	5.23	1.48	—	13
Soybeans, S-100 variety	7.56	2.52	5.32	7.98	6.54	1.42	5.12	3.76	5.32	1.46	—	13
Rapeseed meal	7.2	—	4.5	8.7	5.4	5.3	—	4.8	6.5	—	6.6	14
Rapeseed meal	6.6	—	—	6.9	—	—	1.9	3.3	4.2	1.2	—	15
Rapeseed meal	5.6	2.6	3.7	5.7	3.5	1.1	4.0	3.8	5.7	2.0	2.3	16
Sunflower seed meal	7.76	2.19	4.52	5.95	3.81	2.19	5.12	3.43	4.90	1.38	—	3
Tucum meal	9.47	1.51	3.44	5.95	4.78	1.09	3.10	2.18	3.44	0.84	2.68	2

Tung meal	10.87	2.30	4.93	7.57	4.17	2.24	7.28	4.66	8.33	1.68	—	3
Tung meal	9.0	1.8	4.7	7.5	4.6	1.6	4.1	4.1	7.1	—	—	4
Tung meal	10.5	1.9	4.4	8.0	4.7	2.0	4.6	4.2	7.7	—	—	4
Walnut meal	7.44	1.61	3.22	5.22	2.15	1.23	3.30	2.61	3.82	1.53	—	3
Cereal grains, products, and by-products												
Barley meal	4.6	2.1	3.8	6.8	3.5	1.4	5.3	5.0	5.2	—	—	17
Barley	4.7	1.4	4.1	6.6	3.2	1.2	4.8	3.0	4.9	—	—	18
Barley, malt, diastatic	5.29	2.36	4.23	6.60	3.99	1.46	5.05	3.50	5.70	1.79	—	3
Barley, malted	4.71	1.87	4.56	6.58	4.79	1.05	4.26	3.14	4.26	1.20	3.22	2
Barley, malt sprouts	4.31	1.83	3.76	5.65	4.69	1.34	3.14	3.48	5.03	1.41	—	3
Barley, malt sprouts	4.2	1.9	3.9	5.5	4.5	1.3	2.9	3.4	5.1	1.3	—	19
Brewers' dried grains	4.59	1.75	5.39	8.34	3.28	1.24	4.70	3.20	5.42	1.31	4.11	2
Brewers' dried grains	3.56	2.02	5.72	9.90	3.17	1.26	4.18	3.38	4.21	0.83	3.13	2
Brewers' dried grains	5.0	2.2	4.7	9.7	3.5	1.9	5.8	3.6	5.6	1.3	—	19
Brewers' dried grains	4.87	2.48	5.09	9.56	4.08	1.99	5.36	3.95	5.95	1.12	—	3
Corn	4.8	2.5	6.4	15.0	2.3	3.1	5.0	3.7	5.3	0.6	6.0	20
Corn, low protein	6.1	2.2	3.6	9.3	3.0	2.1	4.1	4.9	7.5	1.2	3.7	21
Corn, high protein	5.3	2.2	3.9	12.6	2.4	1.9	4.8	4.7	7.1	1.0	4.1	21
Corn, white	4.47	2.70	4.35	10.70	4.47	0.94	4.12	3.76	4.47	0.82	5.64	2
Corn, yellow	4.7	2.2	4.4	14.4	2.3	1.4	5.3	3.9	5.3	0.5	—	22
Corn meal	4.4	2.3	—	19.6	2.5	1.9	4.4	4.7	5.4	0.1	—	23
Corn meal	4.18	2.41	4.29	10.77	3.35	1.26	3.97	3.56	4.29	0.73	4.29	2
Corn flakes	4.73	2.69	3.97	13.32	4.40	0.75	3.97	3.33	5.05	0.97	4.94	2
Corn flakes	3.08	1.74	3.08	10.47	2.67	1.03	3.28	2.77	3.90	0.72	4.72	2
Corn germ	8.1	3.0	4.2	7.1	5.8	1.6	5.0	4.4	5.3	1.3	5-6	20
Corn germ meal	6.27	3.02	3.88	8.21	4.74	1.98	4.11	3.88	5.91	1.04	—	3
Corn germ meal	6.65	3.44	4.23	8.68	5.24	1.90	4.19	4.14	6.61	1.28	—	3
Corn gluten	3.1	2.1	5.1	16.0	1.5	2.5	6.6	4.0	5.7	0.6	6.3	20
Corn gluten feed	3.52	2.37	5.14	10.88	3.18	1.26	4.07	3.44	4.51	0.56	3.70	2

TABLE II (Continued)

Foodstuff	Argi- nine	Histi- dine	Iso- leucine	Leucine	Lysine	Methi- onine	Phenyl- alanine	Threo- nine	Valine	Tryp- tophan	Tyro- sine	Ref.
Cereal grains, products, and by-products (<i>continued</i>)												
Corn gluten feed	4.29	3.02	3.54	10.23	3.24	2.10	3.80	3.54	5.51	0.70	—	3
Corn gluten meal	3.28	2.19	4.44	16.47	2.10	2.67	6.12	3.56	5.18	0.52	—	3
Corn hominy, white	4.19	1.97	3.60	8.56	3.85	0.68	3.34	3.17	3.94	0.77	3.60	2
Corn hominy, yellow	5.33	2.27	4.37	8.30	4.11	0.87	3.58	3.67	4.11	0.96	4.02	2
Corn protein (Zein)	1.8	1.7	7.3	23.7	0.0	2.3	6.4	3.0	3.0	0.1	5.2	20
Distillers' dried grains	4.18	2.53	4.37	12.03	3.18	2.15	4.37	3.79	5.83	0.88	—	3
Distillers' dried grains (corn)	4.19	2.37	3.65	8.89	3.11	1.90	4.00	3.41	4.62	0.85	—	3
Distillers' dried grains with solubles	3.53	2.47	4.03	9.86	3.31	2.17	4.51	3.46	5.12	0.81	—	3
Distillers' dried grains with solubles (corn)	4.03	2.70	4.21	10.60	2.70	2.16	4.53	3.88	5.28	0.83	—	3
Distillers' solubles	3.55	2.37	3.59	5.96	2.48	1.78	3.70	3.33	4.37	0.81	—	3
Distillers' solubles	3.63	2.02	3.69	8.48	4.24	1.27	4.24	3.39	4.69	0.82	2.77	2
Distillers' solubles	3.93	2.18	4.79	9.53	4.39	1.38	4.08	3.47	5.54	0.69	3.24	2
Distillers' solubles (corn)	4.29	2.42	4.08	8.73	3.25	1.98	4.55	3.93	5.27	0.90	—	3
Fermentation solubles, corn	2.86	1.62	4.17	8.47	4.70	1.53	3.49	3.83	4.20	0.75	—	3
Fermentation solubles, cane syrup	1.87	1.03	4.50	5.17	3.76	1.63	2.87	3.86	4.81	0.77	—	3
Fermentation solubles, molasses	0.52	0.47	1.53	1.91	0.94	0.73	1.40	1.79	2.29	0.38	—	3
Oatmeal	6.8	2.1	4.4	7.4	3.9	1.5	5.5	3.4	5.7	—	—	24
Oat flour	5.72	1.70	4.38	7.05	4.50	1.03	4.01	3.10	4.38	1.28	3.83	2
Oat, mill feed	4.09	1.12	3.35	5.77	3.53	0.37	2.79	2.60	3.72	0.74	2.79	2
Oat mash	5.44	1.58	4.33	6.70	4.10	0.63	4.18	2.92	4.65	1.26	3.39	2

Rice, white	8.8	2.3	4.4	8.6	2.8	1.4	4.8	3.6	6.4	0.1	—	22
Rice, white	8.3	2.2	4.5	7.8	3.7	2.3	4.5	3.4	6.2	—	—	24
Rice, brown	8.4	2.4	4.6	7.9	3.9	2.1	4.8	3.6	6.2	—	—	24
Rice, brown	8.6	2.5	4.5	9.0	4.5	2.2	4.6	3.2	6.4	1.1	5.8	25
Rice	7.2	1.7	5.2	8.2	3.2	3.0	5.0	3.8	6.2	1.3	5.7	20
Rice bran	9.5	3.1	5.0	7.3	5.9	3.6	4.6	3.9	6.4	3.8	5.2	26
Sorghum, yellow Milo	3.24	1.76	5.18	13.23	2.59	0.46	4.63	3.15	4.72	1.02	4.16	2
Sorghum germ meal	6.84	3.38	4.53	8.70	3.98	1.62	4.62	3.42	6.66	1.02	—	3
Sorghum germ meal	5.68	3.46	4.22	7.84	3.82	1.57	4.03	4.15	6.22	1.16	—	3
Sorghum gluten feed	4.27	2.74	4.40	11.37	3.01	1.66	4.58	3.59	5.80	0.85	—	3
Sorghum gluten meal	2.67	1.82	5.07	16.39	1.32	1.62	5.79	3.01	5.66	1.01	—	3
Sorghum gluten meal	2.13	1.68	5.07	17.17	1.08	1.50	5.63	3.05	3.79	1.17	—	3
Sorghum, Milo, steepwater, 54.7% solids	8.78	3.61	1.85	3.46	2.53	2.35	1.72	5.33	6.06	0.70	—	3
Wheat	4.2	2.1	4.2	6.6	2.7	1.4	4.9	2.9	4.3	—	—	24
Wheat	4.3	2.1	4.0	7.0	2.7	2.5	5.1	3.3	4.3	1.2	4.0	20
Wheat	4.43	1.70	4.68	6.30	3.06	0.68	3.83	2.81	4.00	1.36	3.66	2
Wheat, low protein	4.91	1.74	4.55	6.52	3.60	—	4.20	3.29	4.56	—	2.50	27
Wheat, high protein	5.91	1.76	4.63	6.56	3.26	—	4.42	3.24	4.60	—	2.77	27
Wheat bran	7.5	1.7	4.5	6.5	3.9	1.3	3.0	2.5	4.1	1.3	—	20
Wheat bran	6.28	2.06	3.83	5.54	4.28	0.63	3.14	2.63	3.88	1.48	2.91	2
Wheat flakes	5.32	1.99	3.08	7.97	4.05	0.91	3.74	2.60	4.71	1.21	3.44	2
Wheat flour	3.9	2.2	4.2	7.0	1.9	2.0	5.5	2.7	4.1	0.8	3.8	20
Wheat germ	6.0	2.5	4.5	6.7	5.5	1.3	3.0	6.3	4.3	1.0	3.8	20
Wheat germ	7.41	2.28	3.45	5.95	6.55	1.64	3.96	3.95	5.02	1.07	—	3
Wheat germ	5.90	2.04	4.47	5.47	6.68	1.00	3.34	3.39	4.51	1.26	3.34	2
Wheat gluten	3.68	2.16	4.41	7.04	1.88	1.58	5.31	2.69	5.16	0.88	3.16	2
Wheat gluten	3.76	2.11	4.26	6.98	2.10	1.60	5.53	2.80	5.34	0.76	2.14	2
Wheat gluten	3.68	1.98	4.54	7.20	2.02	1.66	5.04	2.80	4.26	0.77	2.78	28
Wheat middlings	5.56	2.04	4.35	6.66	4.24	0.88	3.69	3.08	3.96	1.32	2.75	2
Wheat red dog flour	5.70	2.04	3.24	6.54	3.50	0.63	2.88	2.77	3.82	1.36	2.93	2

TABLE II (Continued)

Foodstuff	Argi- nine	Histi- dine	Iso- leucine	Leucine	Lysine	Methi- onine	Phenyl- alanine	Threo- nine	Valine	Tryp- tophan	Tyro- sine	Ref.
Forages												
Alfalfa chopped	4.89	1.61	6.17	7.07	6.94	0.84	4.18	3.99	4.63	1.35	3.28	2
Alfalfa leaf meal	4.93	2.18	5.14	7.91	5.55	1.54	5.08	4.62	5.65	2.11	—	3
Alfalfa (Lucerne)	6.9	1.9	—	9.0	7.9	—	4.0	10.0	—	—	6.0	29
Alfalfa meal	3.99	1.70	4.73	6.86	5.59	0.37	4.15	3.83	4.31	1.49	3.03	2
Grass leaves	7.0	2.0	5.0	10.0	5.5	2.5	5-6	5.4	5.0	2.2	5.0	20
Orchard grass	7.9	1.5	—	10.0	7.8	—	3.0	11.0	—	—	5.0	29
Red clover	6.5	1.8	—	9.0	6.7	—	3.0	10.0	—	—	2.0	29
Timothy	4.8	1.7	—	16.0	5.2	—	7.0	10.0	—	—	5.0	29
Leaf meal, beet	4.1	1.3	4.2	6.4	5.4	1.7	5.8	3.8	5.1	1.2	—	30
Leaf meal, broccoli	4.8	1.5	3.2	6.4	4.5	1.8	6.0	3.3	4.5	1.4	—	30
Leaf meal, carrot	4.3	1.9	4.5	7.1	4.5	1.7	6.5	4.4	5.5	1.4	—	30
Leaf meal, celery	4.0	1.5	3.9	6.8	2.4	2.2	4.5	3.4	4.8	1.3	—	30
Leaf meal, corn	3.9	1.3	3.6	6.9	3.2	2.8	5.4	3.3	4.8	1.3	—	30
Leaf meal, kale	5.1	1.6	3.4	6.5	3.1	0.9	4.4	3.5	4.6	1.1	—	30
Leaf meal, lima bean	4.2	1.3	3.6	6.6	3.6	1.2	7.0	4.0	5.0	1.4	—	30
Leaf meal, pea	4.6	1.6	4.4	7.8	4.9	1.0	6.0	4.4	5.7	1.5	—	30
Leaf meal, rhubarb	4.7	1.9	4.0	8.4	5.4	1.0	6.1	4.0	5.3	1.6	—	30
Leaf meal, spinach	4.4	1.3	3.6	6.8	4.7	2.3	4.7	3.9	5.0	1.1	—	30
Leaf meal, turnip	4.5	1.4	3.9	6.8	3.0	2.2	5.3	4.0	4.8	1.3	—	30
Animal products												
Animals, entire	7.3	2.8	—	10.8	6.5	3.0	4.5	4.5	—	1.0	3.2	20
Beef, chopped	7.7	2.9	5.0	7.7	7.2	3.3	4.9	5.4	3.6	1.3	3.4	11
Blood flour	4.31	6.04	1.45	13.85	11.00	1.34	7.21	4.91	9.35	1.28	2.35	2
Blood flour	4.34	6.28	1.52	10.10	9.12	1.17	7.01	4.51	8.59	1.35	2.64	2
Blood meal	4.2	5.6	1.1	11.9	8.8	1.1	7.3	4.1	7.8	1.3	2.2	20

Bone, raw	8.06	1.03	2.65	3.53	4.71	0.70	2.24	2.47	3.02	—	2
Casein	4.2	3.2	7.5	10.0	8.5	3.5	6.3	4.5	7.7	1.3	20
Casein	4.31	3.29	6.88	10.14	9.76	3.50	5.91	4.91	9.61	1.36	2
Casein	4.01	3.10	6.36	10.50	9.16	3.12	5.80	4.55	9.54	1.03	2
Casein	4.1	2.5	6.5	12.1	7.5	3.5	5.2	3.9	7.0	1.2	11
Casein	3.90	3.04	6.35	10.02	8.10	3.25	5.40	4.50	7.36	0.96	28
Egg, whole	6.49	2.07	5.67	8.78	7.24	3.85	5.71	5.29	8.79	1.31	2
Egg, whole	6.6	2.4	7.7	9.2	7.0	4.0	6.3	4.3	7.2	1.5	20
Egg, powdered	6.22	2.11	6.18	9.02	6.13	3.22	5.65	4.94	6.99	1.14	28
Egg, albumin	5.98	2.42	6.45	8.77	7.20	4.16	5.94	4.88	7.45	1.15	28
Egg, albumin	6.1	2.4	7.5	9.4	6.5	5.5	7.5	4.2	6.4	1.5	20
Egg, albumin	5.93	2.45	6.02	8.65	7.66	3.58	6.49	4.91	9.30	1.38	2
Egg, yolk	7.2	1.5	—	—	5.7	3.0	4.4	3.5	—	1.5	20
Liver	6.6	2.5	4.8	8.4	7.0	3.2	6.1	5.3	6.0	1.5	20
Liver, meal	5.98	2.19	4.61	7.96	7.01	1.75	4.27	3.67	6.37	0.88	2
Meat, scrap	6.90	2.60	2.96	6.86	10.03	1.43	3.93	3.42	6.04	0.71	2
Meat, scrap	7.39	1.77	4.71	6.05	6.56	1.34	3.34	3.28	4.33	0.54	2
Meat, scrap	6.19	1.78	4.31	6.61	6.72	1.29	3.33	3.18	4.86	0.55	2
Meat, scrap	7.0	3.5	3.4	8.0	5.6	2.0	5.1	3.9	6.1	0.7	20
Milk, whole	4.2	2.6	7.5	11.0	8.7	3.2	5.5	4.7	7.0	1.5	20
Milk, dry skimmed	3.15	2.45	6.97	9.85	8.42	2.03	4.45	4.30	6.33	1.18	2
Milk, dried whey	2.06	1.18	5.54	9.10	7.48	1.00	2.43	4.86	4.49	1.06	2
Milk, lactalbumin	4.0	2.3	7.5	12.1	10.5	2.6	5.0	6.0	6.6	2.5	20
Milk, β -lactoglobulin	3.0	1.8	6.1	16.1	11.4	3.4	4.5	6.0	6.2	2.0	20
Muscle, animal	7.7	3.3	6.0	8.0	10.0	3.2	5.0	5.0	5.5	1.4	20
Muscle, beef	6.88	3.43	5.37	8.14	10.13	2.58	4.38	5.06	6.97	1.06	2

TABLE II (Continued)

Foodstuff	Argi- nine	Histi- dine	Iso- leucine	Leucine	Lysine	Methi- onine	Phenyl- alanine	Threo- nine	Valine	Tryp- tophan	Tyro- sine	Ref.
Animal products (<i>continued</i>)												
Muscle, beef	6.43	3.25	5.17	7.81	8.59	2.70	3.89	4.45	5.13	1.01	2.96	28
Muscle, beef round	13.50	5.93	3.81	5.15	10.44	1.41	2.28	3.22	3.90	1.11	1.64	31
Tankage	5.8	2.7	3.4	8.6	6.0	2.0	5.0	3.5	5.5	0.7	2.7	20
Marine products												
Crab meal	5.08	1.89	3.59	4.55	4.75	1.59	3.42	3.32	5.21	1.00	4.48	2
Fish meal	6.60	2.21	7.01	7.54	8.85	3.03	4.26	4.49	5.40	0.82	2.75	2
Fish meal, menhaden	6.47	2.66	7.31	8.11	8.73	2.77	4.33	4.72	5.78	1.06	2.59	2
Fish meal, red	7.14	2.06	6.08	7.07	10.99	3.37	3.96	4.00	5.15	0.97	2.63	2
Fish meal, red	6.26	2.24	6.47	9.92	10.45	2.70	4.36	4.41	5.23	1.11	3.04	2
Fish meal, red	6.63	2.00	5.42	6.36	11.05	2.96	3.85	4.05	5.34	0.87	2.70	2
Fish solubles	4.83	1.15	4.12	7.52	7.77	2.21	2.99	2.53	4.73	0.60	1.86	2
Fish solubles	5.50	1.20	5.22	5.53	6.11	2.21	2.70	2.76	4.02	0.68	2.36	2
Yeasts												
Brewers'	5.00	2.00	6.10	7.02	6.98	1.35	3.80	4.70	5.76	1.37	2.72	2
Brewers'	4.5	2.1	4.2	7.1	6.4	1.37	4.4	5.1	5.4	1.05	—	32
Brewers'	4.3	2.8	5.9	7.4	7.5	2.7	4.1	5.5	5.0	1.3	3.6	33
Brewers'	4.0	2.0	5.3	7.0	6.9	1.3	3.8	5.1	5.9	1.5	—	22
Brewers'	5.19	2.11	6.20	7.51	7.81	2.11	5.85	5.21	6.46	1.54	—	3
Brewers'	5.4	1.8	3.8	6.8	8.2	1.1	3.8	3.8	5.3	1.1	3.3	34
<i>Candida krusei</i>	4.4	1.9	4.7	7.5	7.9	1.8	4.3	4.9	5.8	1.1	3.9	34
<i>Hansenula anomala</i> I	5.0	1.6	4.2	7.1	7.7	0.7	3.8	4.5	5.6	1.2	3.4	34
<i>Hansenula anomala</i> II	4.4	1.7	5.8	8.6	8.4	0.9	4.7	4.9	6.5	1.2	4.4	34
<i>Pichia membranaceus</i>	4.2	1.8	5.5	8.0	7.6	1.7	4.3	4.9	6.0	1.1	3.7	34
<i>Rhodotorula rubra</i>	6.6	3.6	3.8	5.9	5.4	0.95	3.1	3.2	4.5	0.8	—	35

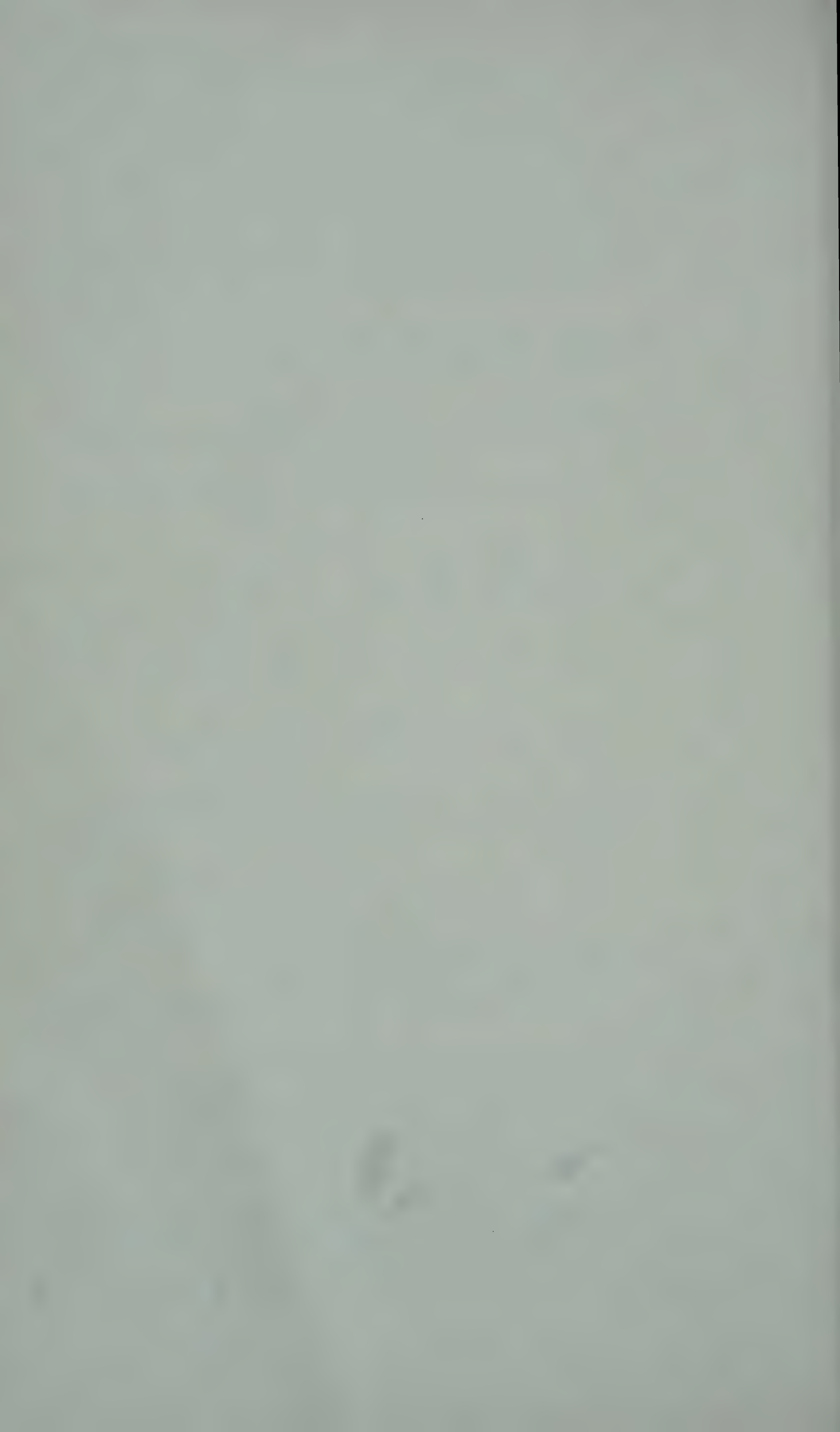
<i>Saccharomyces carlsbergensis</i>	4.4	4.2	4.4	7.2	8.2	1.2	4.0	4.6	5.5	1.1	3.7	34
<i>Saccharomyces cerevisiae</i>	4.3	3.7	4.5	6.8	5.5	1.2	3.8	4.3	5.0	1.1	—	35
<i>Torulopsis utilis</i>	8.6	2.8	5.5	8.3	6.84	2.62	3.59	5.07	6.40	1.63	—	36
Algae												
Commercial preparation	4.00	1.11	4.25	6.09	5.04	1.74	4.87	4.52	5.46	1.29	—	3
Commercial preparation	2.50	0.87	3.16	4.49	2.96	1.25	3.49	3.33	4.06	1.05	—	3
<i>Anabaena cylindrica</i>	11.7	2.5	3.9	6.2	6.6	1.2	2.9	5.7	7.0	1.0	1.6	37
<i>Chlorella vulgaris</i>	15.8	3.3	3.5	6.1	10.2	1.4	2.8	2.9	5.5	2.1	2.8	37
<i>Chondrus sp.</i>	10.2	1.8	—	5.3	4.0	—	1.5	—	2.8	1.6	2.3	37
<i>Cladophora rupestris</i>	5.29	1.07	3.45	5.18	5.69	1.58	3.53	4.30	4.52	1.14	—	3
<i>Cladophora rupestris</i>	6.0	1.1	3.3	5.5	5.6	1.7	3.0	3.9	5.4	0.9	—	4
<i>Fucus vesiculosus</i>	9.4	1.6	3.0	5.0	6.0	0.4	2.6	3.3	3.0	—	1.2	37
<i>Laminaria cloustoni</i>	2.31	1.28	2.57	3.57	3.57	1.37	2.66	4.03	4.30	0.83	—	3
<i>Laminaria cloustoni</i>	2.4	1.0	2.2	3.7	3.0	1.4	2.1	3.6	4.9	0.4	—	4
<i>Laminaria sp.</i>	16.1	1.6	—	2.5	—	—	1.0	—	5.1	1.1	1.9	37
<i>Microcystis aeruginosa</i>	—	—	2.2	4.2	—	1.7	4.4	3.2	4.1	—	—	37
<i>Navicula pelliculosa</i>	9.2	2.8	3.5	7.2	8.3	1.2	3.4	4.2	7.5	1.1	1.9	37
<i>Phormidium sp.</i>	9.2	3.8	—	2.1	—	2.0	1.1	—	6.7	0.2	1.8	37
<i>Rhodymenia palmata</i>	5.22	1.59	4.25	5.41	7.21	1.79	3.91	4.30	6.04	1.30	—	3
<i>Rhodymenia palmata</i>	4.8	1.3	4.0	5.5	6.9	2.3	3.2	4.4	6.4	0.9	—	4
<i>Ulva sp.</i>	7.5	1.2	—	5.2	—	—	2.3	—	5.2	0.3	—	37
Vegetables												
Beans, cranberry	5.53	3.42	6.40	9.06	8.84	1.17	6.45	4.93	6.56	1.48	—	38
Beans, kidney	5.88	2.42	4.93	7.69	9.21	0.99	5.34	3.53	5.92	1.07	2.79	2
Beans, kidney	5.32	3.14	5.51	9.17	7.22	1.20	6.51	4.52	7.13	1.07	—	38
Beans, lima	6.12	3.42	5.99	8.33	5.86	1.46	6.20	4.72	7.77	0.88	—	38
Beans, pink	5.27	3.67	6.44	8.97	6.71	1.16	6.71	5.20	6.44	1.21	—	38
Beans, white, large	6.18	3.34	6.72	8.79	8.70	1.14	6.32	4.35	6.22	1.24	—	38
Beans, white, small	5.83	2.97	6.54	8.48	5.72	1.12	6.80	5.36	6.39	1.35	—	38
Garbanza	9.00	2.61	5.10	7.42	6.34	1.75	6.77	3.69	6.30	0.72	—	38
Lentils	9.26	2.53	4.73	7.67	5.83	0.82	4.57	3.83	6.44	0.82	—	38

TABLE II (Continued)

Foodstuff	Argi- nine	Histi- dine	Iso- leucine	Leucine	Lysine	Methi- onine	Phenyl- alanine	Threo- nine	Valine	Tryp- tophan	Tyro- sine	Ref.
<i>Vegetables (continued)</i>												
Peas, blackeye	7.76	2.98	4.78	10.42	9.61	0.72	5.73	3.79	5.37	1.44	4.74	2
Peas, split, green	10.67	2.46	4.93	7.87	6.64	0.77	4.93	3.96	5.69	0.95	—	38
Peas, split, yellow	10.61	2.41	5.05	8.03	7.12	0.80	5.01	4.07	5.86	0.96	—	38
Potatoes, white	5.0	2.2	3.7	9.6	8.3	2.5	5.9	6.9	5.3	2.1	—	20
Potatoes, sweet	2.9	1.4	3.6	4.8	4.3	1.7	4.3	3.8	5.6	1.8	—	20
Tomato pomace, dried	5.34	1.85	3.26	7.63	7.19	0.22	3.79	3.35	4.41	0.93	4.10	2
Turnip greens	6.26	1.73	4.44	9.31	7.66	0.25	4.86	4.78	6.43	1.86	3.68	2
<i>Miscellaneous</i>												
Acorns	5.88	2.04	4.58	6.60	5.18	1.13	3.86	3.54	5.86	1.02	—	38
Beet pulp, dried	3.65	2.15	4.08	6.01	7.73	0.11	3.01	4.08	4.83	0.97	4.62	2
Citrus pulp	4.81	1.55	3.10	5.33	3.44	1.37	3.09	3.09	4.30	1.03	—	3
Coffee pulp	3.68	1.98	3.78	5.48	3.40	2.64	3.12	3.12	4.72	0.94	—	3

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APPENDIX

NATIONAL AVERAGE FOOD SUPPLIES

There is presented in the following tables and figures information on the distribution of animal and vegetable protein in national diets. It must be recognized that these estimates are for average consumption; they do not show the range of variation within a nation. It is quite likely that substantial groups in each nation have available to them quantities of protein above and below the average figures.

TABLE I

ESTIMATED PER CAPUT CONSUMPTION OF ANIMAL AND VEGETABLE PROTEIN
IN COUNTRIES WHICH MAINTAIN FOOD BALANCE SHEETS^a

Country	Population (1953)	Protein consumption	
		Animal	Vegetable
	thousands	g. per caput per day	
Far East			
Burma	19,045	28	37
Japan	86,700	13	45
Ceylon	8,155	11	32
India	372,000	6	41
Pakistan	75,842	11	38
Philippines	21,039	10	33
Near East			
Cyprus	506	16	50
Egypt	21,935	11	58
Israel	1,650	26	62
Turkey	22,461	14	72
Eritrea	1,104	11	39
Africa			
French Morocco	8,220	18	64
Union of South Africa	13,153	29	46
Southern Rhodesia	2,260	16	65
Latin America			
River Plate countries			
Argentina	18,393	57	39
Uruguay	2,525	66	34

TABLE I (Continued)

Country	Population (1953)	Protein consumption	
		Animal	Vegetable
Others			
Brazil	55,772	16	41
Chile	6,072	26	51
Colombia	12,108	30	26
Mexico	28,053	15	50
Peru	9,035	12	42
Venezuela	5,497	21	37
Dominican Republic	2,291	6	32
El Salvador	2,052	4	45
Guatemala	3,049	13	43
Honduras	1,564	18	57
Europe			
Mediterranean			
Greece	7,819	19	59
Italy	47,756	22	53
Portugal	8,621	21	50
Spain	28,528	21	57
Eastern			
Yugoslavia	16,991	17	61
Western			
Austria	6,954	38	44
Belgium/Luxembourg	9,082	40	46
France	42,860	43	51
Ireland	2,942	47	48
Netherlands	10,493	41	39
Switzerland	4,877	50	43
United Kingdom	50,857	44	41
Fed. Republic of Germany	48,994	38	39
Northern			
Denmark	4,369	53	40
Finland	4,141	43	51
Norway	3,359	53	43
Sweden	7,171	59	33
North America and Oceania			
Canada	14,871	59	36
United States of America	159,629	63	29
Australia	8,876	63	30
New Zealand	2,047	69	33

^a Prepared by C. J. Amaral and furnished through the courtesy of A. G. van Veen, Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, April, 1956.



FIG. 1. Caloric content of national average food supplies. [From *Food and Agr. Organization U.N., Second World Food Survey* p. 23 (November, 1952).]

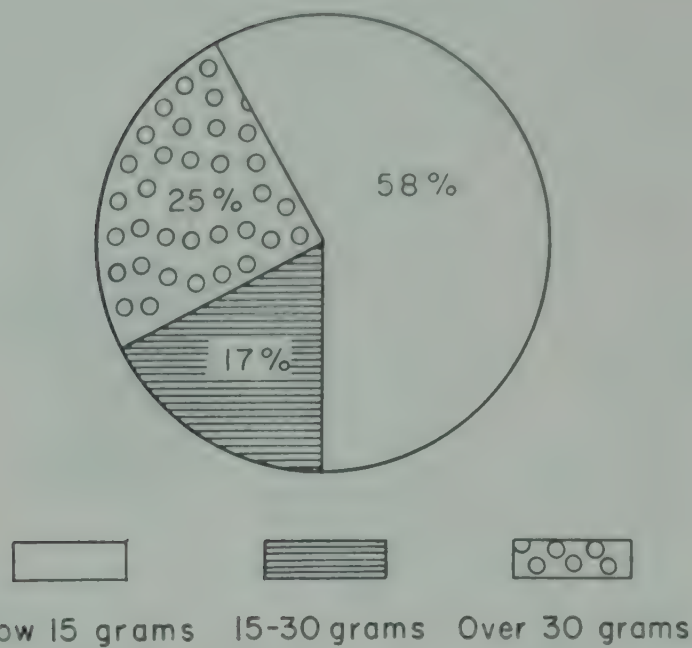


FIG. 2. Distribution of world's population according to daily average supplies of animal protein. [From *Food and Agr. Organization U.N., Second World Food Survey* p. 24 (November, 1952).]

TABLE II
ESTIMATED PROTEIN CONSUMPTION OF WORLD POPULATION^a

Region	Population—1953			Protein consumption in countries with F.B.S.		Estimated protein consumption of total population	
	Countries with F.B.S. ^b	Total	Percentage reporting				
	(1)	(2)	(1)/(2)	Animal	Vegetable	Animal	Vegetable
	millions	millions	%	thousand metric tons per year		thousand metric tons per year	
Far East	582.8	1236.6	47	1,775	8,640	3,775	18,380
Near East	47.7	132.3	36	222	1,115	615	3,100
Africa	23.6	162.6	15	206	464	1,375	3,090
Latin America							
River Plate countries	20.9	22.4	93	444	292	480	316
Others	125.5	150.8	83	790	1,929	950	2,320
Europe							
Mediterranean	92.7	93.5	99	722	1,930	729	1,949
Eastern	17.0	89.1	19	105	378	550	1,990
Western	177.1	198.5	89	2,581	2,793	2,900	3,140
Northern	19.0	19.2	99	369	280	375	284
North America and Oceania	185.3	188.6	98	4,244	2,006	4,330	2,050
Total	1291.6	2295.2 ^{c,d}	56	11,458	19,827	16,079	36,619

^a Prepared by C. J. Amaral and furnished through the courtesy of A. G. van Veen, Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, April, 1956.

^b Countries which maintain food balance sheets.

^c Excluding U.S.S.R.

^d World total does not coincide with regional totals because it includes allowances for those countries for which no estimates were available for the year considered.

AUTHOR INDEX

Numbers in parentheses are reference numbers and are included to assist in locating the references where the authors' names are not mentioned on the page. Numbers in *italics* refer to the page on which the reference is listed.

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